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Sixty-fourth Annual Meeting of the American Radium Society
Sixth Annual Meeting of the Cell Kinetics Society

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

Nodular Poorly Differentiated Lymphocytic Lymphoma: Changes in Histology and Survival [1]

Stanley S. Ostrow, Charles H. Diggs, John C. Sutherland, JoAnn Gustafson, and Peter H. Wiernik [2,*]

SUMMARY

Lymphoma patients with a nodular pattern have a better prognosis than those with a diffuse pattern. Histologic evolution from nodular to diffuse may occur during the course of the disease in the same patient. This change in pattern may be of prognostic significance. We reviewed 56 patients with an initial diagnosis of nodular poorly differentiated lymphoma seen over a 12-year period (1966–1978). Thirty-five patients had biopsies after initial diagnosis, and 28 of the cases could be classified as nodular or diffuse. Eleven of the 56 cases evolved from a nodular to a diffuse pattern. Forty-four of the 56 patients achieved a complete remission and were evaluated for survival. The median survival (66+ months) for patients who have never relapsed is similar to that for patients who relapsed with a nodular pattern (70 months) and patients who relapsed with a diffuse pattern (73 months). Relapse with a nodular pattern occurred at a median of 27 months after initial diagnosis and relapse with a diffuse pattern occurred at a median of 49 months. Although overall survival in patients relapsing with nodular or diffuse disease is similar, median survival from relapse with nodular disease is longer (32+ months) than median survival with diffuse disease (17 months) ($P = 0.068$).

[Cancer Treat Rep 65:929–933, 1981]

Both cell type and histologic pattern are of prognostic importance in patients with non-Hodgkin's lymphoma (7–9,13). Patients with nodular poorly differentiated lymphocytic (NPDL) lymphoma have been demonstrated to have a greater rate of response to chemotherapy and a better survival than those with a diffuse PDL (DPDL) lymphoma (7). Patients who present with a cell type of PDL and a histologic pattern of nodular and diffuse have a long-term and disease-free survival similar to that of patients who present with a PDL lymphoma of only nodular pattern (22).

Patients who have a nodular pattern at initial biopsy may exhibit histologic evolution to a diffuse pattern during the course of their disease (11,12,19). The question of whether a change in histologic pattern from nodular to diffuse in the same patient influences survival has not been answered. The purpose of this study was to investigate this question by studying patients with an initial diagnosis of NPDL lymphoma and comparing those whose histologic pattern changed to DPDL on rebiopsy to those who either continued to show a nodular pattern on rebiopsy or did not relapse.

METHODS

The records of 56 patients with an initial diagnosis of NPDL lymphoma who were admitted to the Baltimore Cancer Research Program between January 1966 and December 30, 1978, and in whom followup has been maintained were reviewed.

All pathologic material from admission, relapse,

[1]Received May 7, 1980; revised Mar 2, 1981; accepted Mar 24, 1981.

[2]Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute, MD.

**Reprint requests to:* Peter H. Wiernik, MD, Baltimore Cancer Research Program, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

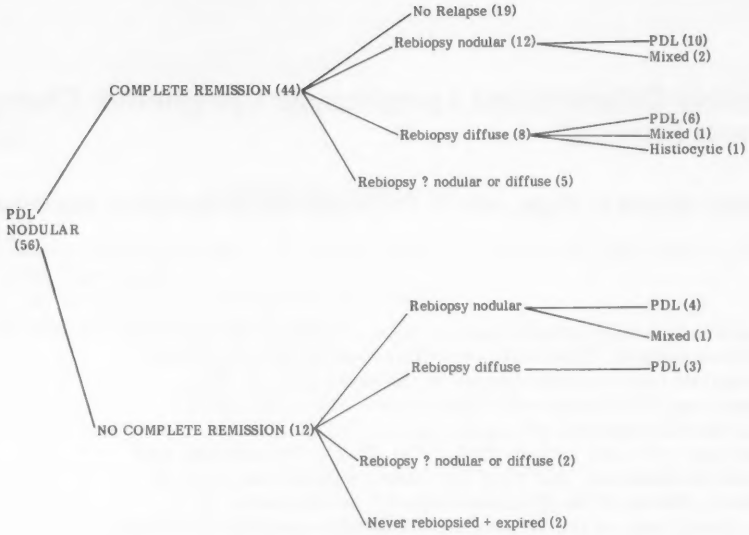

FIGURE 1.—Results of rebiopsy in 56 patients with an initial diagnosis of NPDL lymphoma.

and post-mortem examination was reviewed by a hematopathologist. Classification was by the method of Rappaport (20). Staging was by the Ann Arbor staging criteria (4).

Treatment regimens varied with the protocol in use at the time of admission. These included high-dose cyclophosphamide alone (16); total-body irradiation (3); or mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) between 1966 and 1971 (14); cyclophosphamide, vincristine, and prednisone (COP) or MOPP between 1971 and 1973 (1); and COP or cyclophosphamide, doxorubicin, vincristine, and prednisone between 1973 and 1979 (6).

Some patients had received their initial therapy prior to referral to the Baltimore Cancer Research Program. These patients had received localized irradiation, total-body irradiation, both chemotherapy and radiotherapy, or chemotherapy alone.

Statistical Analysis

Comparison by groups was performed by the Wilcoxon test as described by Gehan (10). Actuarial survival was calculated by the method described by Cutler and Ederer (5).

RESULTS

Twenty-nine males and 27 females were studied. The median age at diagnosis was 52 years (range, 25–71).

Figure 1 shows the results of rebiopsies of the 56 patients whose initial biopsy indicated NPDL lymphoma. Biopsies were performed at the time of suspected disease progression. Thirty-five patients had rebiopsies, and 28 of the cases could be classified as nodular or diffuse. The other seven patients relapsed in areas (ie, bone marrow) where a determination of nodular or diffuse pattern could not be made. In 11 patients a nodular pattern had changed to a diffuse pattern. The frequency of documented change in histologic pattern for the total group was 20% (11 of 56 patients). For those patients who have had rebiopsies where a determination of pattern could be made, the frequency of change was 40% (11 of 28 patients). Most of the 28 patients who have not changed histologic pattern are still alive and at risk for relapse.

Table 1 compares three groups of patients who had an initial diagnosis of NPDL lymphoma and have achieved complete remission. Group 1 patients have never relapsed, group 2 patients have relapsed with a nodular histology, and group 3 patients have relapsed with a diffuse histology. Two of the patients in group 3 had prior biopsies with NPDL lymphoma.

Most of the patients in all three groups had stage III or IV disease at initial presentation. Combination chemotherapy was used as initial therapy in 63% of patients who have never relapsed (group 1), in 33% of patients who have relapsed with a nodular pattern (group 2), and in 50% of patients who have

TABLE 1.—Patient characteristics and response*

Characteristic	Group 1	Group 2	Group 3
Median age at diagnosis in yrs (range)	52 (32–71)	45 (25–68)	54 (29–68)
Sex			
Males	10	8	4
Females	9	4	4
Stage at diagnosis			
I	2	3	1
II	3	1	0
III	7	1	1
IV	7	7	6
Median survival in mos (range)	66+ (9–156+)	70+ (23–196)	73 (46–159)
No. alive (July 1, 1979)/ No. of patients	16/19	8/12	1/8
Median time from diagnosis in mos (range)			
To rebiopsy		27 (10–89)	
To change in histology			49 (21–159)
Median survival from rebiopsy in mos (range)		32+ (11–90)	17 (6–57+)†

*Unless specified otherwise, values = No. of patients.
†Does not include 2 patients who had change in histology first documented at post-mortem examination.

relapsed with a diffuse pattern. One patient in group 1, one in group 2, and none in group 3 received single-agent chemotherapy initially. Radiation therapy with or without chemotherapy was used in 30% of patients in group 1, in 50% of group 2, and in 50% of group 3 as initial therapy.

Table 1 shows median time to change in histology for group 3 and the range of survival from rebiopsy for groups 2 and 3. In patients who continued to show a nodular pattern at rebiopsy, the median time from diagnosis to rebiopsy was 27 months. In patients whose histology changed to diffuse, the change occurred well into the course of the disease (median, 49 months).

Survival from time of diagnosis as shown in figure 2, using Wilcoxon analysis, reveals no significant differences among the three groups (group 1 vs 2, $P = 0.871$; group 1 vs 3, $P = 0.461$; and group 2 vs 3, $P = 0.644$).

The actuarial survival from rebiopsy reveals a trend toward a significant difference between groups 2 and 3 ($P = 0.068$), with those who relapsed with a nodular pattern (group 2) having a longer survival than those who relapsed with a diffuse pattern (group 3).

Twelve patients did not achieve a complete remission prior to rebiopsy. Only two of these 12 patients ever achieved a complete remission at any time in the course of their disease. Only one patient was alive at the end of the followup period (July 1979); the median survival from time of diagnosis of these 12 patients was 38 months.

DISCUSSION

This study examined change in histology and survival data for 56 patients with an initial diagnosis of NPDL lymphoma. The most frequent change in histology was to a diffuse pattern. This change was seen in 20% of the overall sample and in 40% of those patients in whom a rebiopsy was performed and a determination of histologic pattern could be made.

The reported frequency of change from nodular to diffuse lymphoma had varied in earlier studies (15,21). This variation may have been due to differing criteria for nodular ("follicular" in the older literature) lymphoma. Wright (23), in a study of macrofollicular lymphoma, found that only eight of 30 patients with multiple rebiopsies showed persistence of a follicular pattern. Patterns of diffuse histology were noted in all 21 patients with macrofollicular lymphoma who had post-mortem examination (23). Lumb and Newton (15), in their analysis of 602 cases of lymphoid tumor, identified 35 cases with an original diagnosis of follicular lymphoma. Nine of 12 patients who had post-mortem exami-

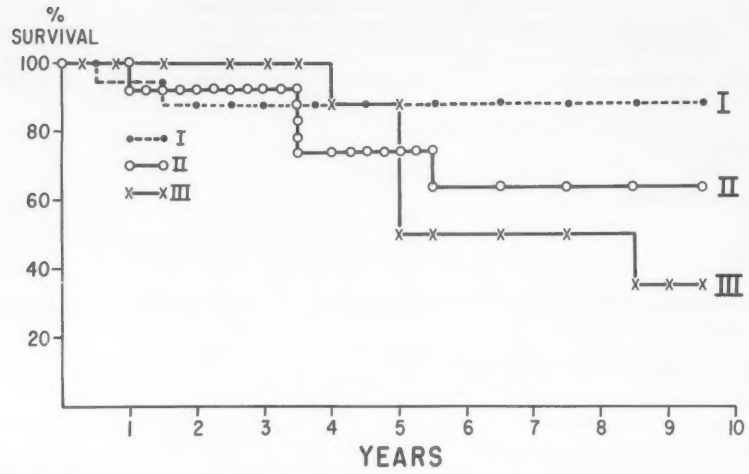

FIGURE 2.—Actuarial survival from initial diagnosis in patients who never relapsed (group 1) compared to patients who relapsed with nodular (group 2) or diffuse (group 3) histologic patterns.

nation retained their follicular lymphoma. Several of their patients who underwent rebiopsy had a breakdown at the edges of the nodular lymph node, suggesting a transition to a diffuse pattern. These patients were noted to have lived for a number of years following their diagnosis.

Rappaport et al (21) reported a change in histology in 24 of 46 patients with an initial diagnosis of poorly differentiated follicular lymphoma. The most frequent change was from nodular to diffuse, with no change in cell type. Most patients in their series were treated with irradiation. The frequency of change in histologic pattern in their series (52%) is similar to that in ours (40%) for those patients who underwent rebiopsy where a determination of histologic pattern could be made. In our series most patients received combination chemotherapy during the course of their disease. The similarities in frequency of change of histology may indicate that therapy does not affect change in histology to any significant degree. Two recent studies compared survival of patients who retained their nodular pattern on rebiopsy to those who changed to a diffuse pattern. Qazi et al (19), examining overall survival in patients with non-Hodgkin's lymphoma who underwent post-mortem examination, found that patients with a nodular pattern had a median survival of 55 versus 32 months in patients with evolution to diffuse disease. Jones et al (11,12) reported an overall median survival of 79 months for patients with nodular lymphoma versus 46 months for patients whose nodular disease converted to mixed diffuse or histiocytic diffuse. Following conversion to diffuse histology the median survival was 8 versus 73 months in patients with nodular lymphoma whose histology remained unchanged (11,12).

The question of when to treat patients with nodular lymphoma continues to be debated. A recent report indicates that long-term survival is seen in patients who are asymptomatic with stage III and IV nodular (mixed or PDL) lymphoma who are not initially treated (17). Another report indicates that in patients with nodular lymphoma, histiocytic cell type, B symptoms, advanced age, central nervous system, or extensive intra-abdominal involvement are associated with short survival (18). In that report, patients with a diffuse component did not have a significant difference in survival compared to those with an exclusively nodular pattern.

It is not clear whether a change in histologic pattern is an indication for a change of therapy. In our series of patients, therapy was given to patients whether or not they were symptomatic and regardless of the stage of disease. Although changes in histologic pattern on rebiopsy were not associated with significant differences in survival from the time of rebiopsy in our patients, there was a trend to longer survival for those patients relapsing with nodular compared to diffuse histology. This would seem to indicate that a change in histologic pattern may adversely affect prognosis. Bloomfield et al (2) indicate that a combination of histology and lym-

phocytic surface markers has prognostic significance. Comparing these markers at diagnosis and at relapse may identify groups with different survival patterns. The previously mentioned factors as well as further subdivisions of nodular lymphomas by immunologic parameters may provide the clinician with a clearer distinction in terms of prognosis and response to therapy.

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Histologic Variation in Non-Hodgkin's Lymphomas: Commentary[1,2]

Richard T. Hoppe[3,4,*]

One of the most intriguing aspects of the non-Hodgkin's lymphomas is the variation in histology displayed by some patients either at presentation or during the course of their disease. Several types of histologic variation have been analyzed recently in the literature, including (*a*) mixed architectural pattern (a single biopsy showing nodular and diffuse areas), (*b*) composite lymphoma (variation of cytologic type within a single biopsy), (*c*) discordant (divergent) lymphoma (variation in histologic subtype in different sites at a single time), (*d*) histologic "conversion" (or "evolution") (a new histologic subtype that becomes apparent during the course of a patient's disease), and (*e*) histologic change documented only at autopsy. In this commentary I shall discuss briefly each of these forms of histologic variation, with a more detailed consideration of histologic conversion.

Mixed Architectural Pattern

A mixed architectural pattern designates those instances in which a single biopsy reveals a follicular (or nodular) pattern with diffuse areas; however, a constant cell population is present throughout [ie, poorly differentiated lymphocytic, histiocytic, or mixed histiocytic-lymphocytic in the Rappaport system (1)]. Lymphoma with a mixed architectural pattern is observed frequently and data have now been reported from several centers regarding its frequency and impact on prognosis. The reported proportion of patients with nodular poorly differentiated lymphocytic lymphoma (or its equivalent in other systems) who have diffuse areas in the same biopsy ranges from 17% to 45% (2–5). Initial clinical studies suggested that the presence of diffuse areas in a biopsy had little impact on prognosis (2,6). However, recent reports with more patients and longer followup reveal a worse survival for patients with a mixed architectural pattern compared to those with a purely follicular lymphoma (3–5).

Table 1 summarizes the survival and freedom-from-relapse data for a large group of patients with follicular lymphoma (with or without diffuse areas) who were part of a multi-institutional study (5).[5] These patients all had a diagnosis of follicular, small cleaved cell lymphoma according to the criteria of Working Formulation of Non-Hodgkin's Lymphoma for Clinical Usage.[5] In the Rappaport system this entity corresponds most closely to nodular poorly differentiated lymphocytic lymphoma. This analysis was restricted to patients with pathologic stage III–IVA disease (7) since both stage and the presence of systemic symptoms influence prognosis. Patients were treated between 1971 and 1975 at four major centers (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy; Stanford University; The Tufts-New England Medical Center; and the University of Minnesota). Rates of initial complete response and median relapse-free survival were not different; however, there was a significant survival advantage favoring the group of patients with purely follicular lymphoma ($P = 0.01$). This suggests a more aggressive behavior for lymphoma with a mixed architectural pattern once relapse has occurred and may indicate the need for more aggressive initial therapy in these patients.

Composite Lymphoma

True composite lymphomas demonstrate variation in cell type within a single biopsy (8). There should be clear demarcation between areas of different cell types, thereby excluding cases of mixed histiocytic-lymphocytic lymphoma, which display an intimate admixture of different cells. Kim et al (8,9) reported 20 cases of composite lymphoma and an incidence of 3.5% among patients with non-Hodg-

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[3]Division of Radiation Therapy, Department of Radiology, Stanford University Medical Center, CA.

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Reprint requests to: Richard T. Hoppe, MD, Division of Radiation Therapy, Department of Radiology, Stanford University Medical Center, Stanford CA 94305.

[5]Non-Hodgkin's lymphoma classification project: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a working formulation for clinical usage. Submitted for publication.

TABLE 1.—Response and survival data for patients with follicular, small cleaved cell lymphoma with or without diffuse areas

	Rates (%) for —		
	Follicular lymphoma	Follicular and diffuse lymphoma	*P* value
Complete response	75	67	0.4
5-yr freedom-from-relapse	34	28	0.2
5-yr survival	83	54	0.01

kin's lymphoma at Stanford University. In the multi-institutional study cited above (footnote 5), the frequency of composite lymphoma varied according to the pathologist and classification system but ranged from 1.0% to 4.7% of > 1000 cases.

The natural history of composite lymphoma has not been defined clearly. At Stanford University, our clinical impression has suggested that the tempo of disease is determined by the more "aggressive" component of lymphoma, and our treatment policies have evolved accordingly (9).

Discordant Lymphoma

By definition, the diagnosis of discordant lymphoma depends on examination of multiple biopsies. Therefore, this entity was described well only after the introduction of surgical staging for the non-Hodgkin's lymphomas (9). Many cases of discordant lymphoma demonstrate marrow infiltration by small cleaved cells (poorly differentiated lymphocytes), while nodal or other tissue biopsies show mixed cell or predominantly large cell populations. Goffinet et al (10) reviewed the histologic findings in a large group of patients with non-Hodgkin's lymphoma who underwent routine surgical staging procedures, often including laparotomy with selected lymph node biopsies and splenectomy. They identified discordance of histology in 20% of patients with lymphoma in two or more biopsy specimens. A more recent study from the National Cancer Institute has shown discordance in 33% of patients with multiple biopsies (11).

The natural history of discordant lymphomas is also undefined. Again, clinical observations suggest that these patients have a course consistent with the more "aggressive" type of lymphoma, and therefore at Stanford University, our treatment is planned accordingly (10). The recent study from the National Cancer Institute, however, indicates that these patients have an intermediate prognosis (11).

Histologic "Conversion" or "Evolution"

In the non-Hodgkin's lymphomas, a true "evolution" of histologic type during the course of the disease is analogous to the Richter syndrome (12). In Richter's syndrome, patients with chronic lymphocytic leukemia develop a large cell (diffuse histiocytic) lymphoma late in the course of their disease. In the non-Hodgkin's lymphomas, this evolution may be restricted to changes in cell type or architectural pattern, but may include both. Cases also have been observed in which sequential changes in architectural pattern and cell type are documented.

Change in Histology Apparent Only at Autopsy

Histologic change occasionally is detected only at autopsy. These patients may have had a long course of disease with documentation of constant histologic subtype while alive, but careful evaluation of tissues at post mortem examinations reveals changes in cell type or architectural pattern. This type of change may be observed in 63%–100% of patients who presented initially with follicular lymphomas (1,13–15).

DISCUSSION

Among the different manifestations of histologic variation in the non-Hodgkin's lymphomas, the most controversial is histologic conversion. Questions that have been raised regarding this phenomenon include the following. (*a*) Do all follicular lymphomas ultimately demonstrate changes in architectural pattern and/or cell type? (*b*) Does histologic conversion always represent an evolution of disease or are some cases coincidental or treatment-related? (*c*) Do certain therapies either prevent or predispose to histologic change? (*d*) What is the natural history of disease and appropriate therapy once histologic conversion occurs?

Histologic conversion does not occur in every case of follicular lymphoma. For example, the long-term

freedom from relapse for patients with pathologic stage I or II follicular lymphoma treated with irradiation alone is nearly 50%.[6] Histologic change is not observed in these patients when the initial therapy adequately eradicates all disease. However, in patients with pathologic stage III–IV lymphoma, relapse rates are very high, and many of these patients will demonstrate histologic conversion at some time during the course of their disease.[7]

The actual risk for histologic conversion among patients with follicular lymphoma is difficult to measure. A number of studies have addressed this issue and have reported that it occurs in 10%–61% of patients (1,13,16–20). Ostrow et al (21) report a change in histologic pattern in 20%–40% of patients with an initial diagnosis of nodular poorly differentiated lymphocytic lymphoma.

The wide range of values reported for histologic conversion may result from differences in initial staging studies, variable duration of followup, differences in policy regarding rebiopsy at relapse, sites selected for biopsy, and variable inclusion of autopsy data. Series in which only single initial biopsies were obtained may overestimate the rate of subsequent histologic change. On the other hand, policies of infrequent rebiopsy after relapse may underestimate the likelihood of conversion. This is especially true when bone marrow biopsy is used to document recurrence, since a determination of architectural pattern is impossible to make based upon marrow involvement. In addition, selective biopsy of "accessible sites" ignores the possibility that histologic conversion may have occurred at an inaccessible site.

The calculated frequency of conversion also will vary with the size of the denominator group. Some series consider the risk group to include all patients with follicular lymphoma, some restrict it to patients who are rebiopsied at relapse, and a few restrict it to patients who relapse and are rebiopsied from a site where architectural pattern can be defined. The calculated frequency of histologic conversion will increase as this denominator group is reduced. It is imperative to be aware of these influences when evaluating reports of this phenomenon.

Since histologic conversion is often a late event, its true likelihood may only be accurately determined in series with long followup. Variation in followup duration may be adjusted for by using actuarial calculation techniques; however, this type of analysis has been utilized in only one study (footnote 7). These actuarial risk curves indicate that patients who are not cured of their lymphoma have an increasing risk of conversion with time.

While the clinical utility of the Rappaport classification system is well established (22,23), it is difficult to reconcile all aspects of histologic variation with the concepts first proposed in this system. Rappaport suggested that progression from a nodular to diffuse pattern represented the infiltration of neoplastic cells between nodules, ultimately obliterating the nodular pattern. He suggested that when this diffuse infiltration occurred, it signaled transition to a more aggressive clinical behavior of the disease. It is likely that a mixed architectural pattern represents the first phase of this process. However, Rappaport indicated that changes in architectural pattern were not usually accompanied by changes in cytologic type and that composite lymphoma probably represented the coincidental development of two independent lymphomas (22).

More ready explanation for these aspects of histologic variation accompanied subsequent proposals that all follicular (and many diffuse) lymphomas were derived from common precursor cells of the follicular (germinal) center (24–26). Apparent changes in cytologic type could then be attributed to different manifestations of a disease arising from a common precursor cell (27).

Available evidence does confirm that many examples of composite lymphoma and histologic conversion represent the evolution of a single clone of cells. Both cell surface marker studies and cytogenetic analysis of serial biopsies have documented monoclonality in both the Richter's syndrome and examples of histologic conversion (27–31). However, more prospective data are required to extend these observations.

Despite these confirmatory examples, it is unlikely that all cases of histologic change represent an evolution of disease. Some of these new lymphomas are probably treatment-related. Diffuse undifferentiated or large cell lymphomas, apparently related to therapy, have been reported in patients treated for Hodgkin's disease (32). Since treatment programs for Hodgkin's disease and the non-Hodgkin's lymphomas are similar, some cases of histologic "change" probably represent therapy-induced secondary lymphomas. This contention is supported further by the observation that lymphomas frequently develop in immune deficiency states or in patients receiving immunosuppressive therapy for organ transplantation (33).

While lymphoma therapy may result in the development of unrelated secondary lymphomas, there

[6]Paryani S, Hoppe RT, Cox RS, et al. Analysis of non-Hodgkin's lymphomas with nodular and favorable histologies, stage I and II. Submitted for publication.

[7]Acker B, Hoppe RT, Cox RS, et al. Histologic conversion in the non-Hodgkin's lymphomas. Submitted for publication.

is little evidence to suggest that any therapies predispose to histologic conversion. In a study recently completed at Stanford University, the likelihood of histologic conversion was similar for patients with nodular lymphomas treated with either a single alkylating agent, combination chemotherapy (CVP), total lymphoid or whole-body irradiation, or various combinations of chemotherapy and irradiation (footnote 7). However, an important question remains unanswered: Is the likelihood of histologic conversion any different in the patient with nodular lymphoma who is simply followed until symptoms requiring therapy develop? In the only group of these patients reported, rebiopsy prior to initiation of therapy was not usually accomplished (34). Prospective clinical trials currently under way at the National Cancer Institute and Stanford University should be helpful in evaluating this point, since patients with advanced-stage nodular lymphoma may be randomized to receive no initial therapy, with rebiopsy when treatment is eventually required.

The final point of interest regarding histologic conversion relates to the natural history of disease once conversion has occurred. There is general consensus that histologic conversion is an unfavorable prognostic sign, usually accompanied by a more aggressive clinical course than lymphomas that remain nodular on biopsy (18–22,35–37, footnote 7). However, the selection of appropriate treatment for these patients remains elusive. Since multiagent chemotherapy has proven successful in the management of many patients with de novo unfavorable lymphoma (38), similar therapy is usually employed in patients after histologic conversion. However, extensive prior therapy often compromises the adequacy of treatment. In addition, the few series that have been published draw different conclusions regarding the impact of achieving a complete remission after histologic conversion; some find that it favorably influences subsequent prognosis (19,21, footnote 7), while others find it to be of no benefit (20,37).

Since major therapeutic decisions in the non-Hodgkin's lymphomas are based upon consideration of histologic subtype, the concepts of histologic variation are extremely important to the clinical oncologist. Likewise, the pathologist is interested in this phenomenon since it has provided important insights into the natural history and evolution of these diseases. Hopefully, future clinical trials and the application of modern diagnostic aids such as cell surface marker studies will permit us to further our understanding of histologic variation in the non-Hodgkin's lymphomas and permit us to utilize this information effectively in treatment decisions.

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Cyclophosphamide, Doxorubicin, and Cisplatin in the Treatment of Advanced Non-Small Cell Lung Cancer[1]

James A. Knost,[2,3] F. Anthony Greco,[2] Kenneth R. Hande,[2] Ronald L. Richardson,[2] Mehmet F. Fer,[2] and Robert K. Oldham[2,*]

SUMMARY

Fifty-four patients with non-small cell lung cancer were treated with a combination of cyclophosphamide, doxorubicin, and cisplatin (CAP). A 35% response rate was seen, with a median survival of 380 days for the responders, compared to median survival times of 150 days for the nonresponders and 229 days for the group. Twenty-four percent of these patients had received prior treatment. All of the nonresponding patients had died by 438 days, whereas eight of 19 responders lived > 425 days. The survival curve for the nonresponders was similar to that for a historical control group not given chemotherapy. These data indicate that this drug regimen has some efficacy in the treatment of non-small cell lung cancer.

[Cancer Treat Rep 65:941–945, 1981]

Non-small cell lung cancer (NSLC) accounts for 80% of lung cancers. Although surgical resection is the treatment of choice for localized NSLC, nearly 90% of these patients will eventually be candidates for systemic chemotherapy. At least one half of the patients with NSLC have metastatic disease at the time of presentation, and an additional 35%–40% have progressive disease after initial surgery or radiation therapy (1). Treatment of NSLC with chemotherapy has been difficult and no strikingly effective regimen has yet been developed.

Because of the animal data indicating the possibility of synergism when cyclophosphamide, doxorubicin, and more recently, cisplatin are used in combination (2–5) and because of the initial clinical trial from the Mayo Clinic, Rochester, MN (6), we initiated a study of cyclophosphamide, doxorubicin, and cisplatin (CAP) in patients with unresectable advanced NSLC.

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[2]Division of Oncology, Department of Medicine, Vanderbilt University Hospital, Nashville, TN.

[3]*Present address*: Biological Response Modifiers Program, National Cancer Institute-Frederick Cancer Research Center, MD.

**Reprint requests to:* Robert K. Oldham, MD, Biological Response Modifiers Program, National Cancer Institute-Frederick Cancer Research Center, Bldg 426, Frederick, MD 21701.

PATIENTS AND METHODS

Fifty-seven patients with advanced NSLC were entered in this study. The patients were considered evaluable for the assessment of antitumor activity and survival if they completed two cycles of chemotherapy. Patients were eligible if they met the following criteria: < 80 years old, no significant congestive heart failure, wbc count ≥ 3000/mm^3, platelet count ≥ 100,000/mm^3, creatinine clearance ≥ 60 ml/minute, serum creatinine ≤ 2 mg/dl, performance status of 2 or better (Eastern Cooperative Oncology Group [ECOG] performance scale), and a projected life expectancy of at least 6 weeks. Patients who had received prior chemotherapy were excluded from this study. Three patients were considered inevaluable for response because they received only one cycle of chemotherapy.

Prior to therapy, these patients were classified by cell type, age, sex, prior therapy, performance status, and distribution of disease. Of the 54 patients, 21 had locally advanced disease without *clinical* evidence of metastases at presentation. The ratio of males to females was 43:11, median age was 59 years, and 13 patients (24%) had received prior radiotherapy.

Indicator lesions were considered either measurable or evaluable. Measurable lesions were de-

fined as having clear margins, to determine two maximum perpendicular diameters. Evaluable lesions were defined as being visually assessable by radiography but not easily measurable, most often due to their contiguity with mediastinal structures (6,7).

Complete response and partial response (PR) were defined in the usual way (7). Evaluable responses were judged by two investigators and usually involved a clear decrease in lesion size, as shown in chest roentgenogram. CAP was administered iv as follows: cyclophosphamide, 400 mg/m^2; doxorubicin, 40 mg/m^2; and cisplatin, 40 mg/m^2. The drugs were given six cycles of CAP if they had stable disease 120 minutes, with the administration of 1 liter of 5% glucose in one-half normal saline. Patients were given six cycles of CAP if they had stable disease or if they showed response. No maintenance therapy was given after CAP. Cyclophosphamide and doxorubicin were given with the following dose modifications: wbc count $\geq$ 3500/mm^3, 100% of the dose; wbc count of 3000–3500/mm^3, 75%; wbc count of 2500–3000/mm^3, 50%; and wbc count $\leq$ 2500/mm^3, treatment withheld. Doses were decreased to 50% if the platelet count was 100,000–150,000/mm^3, and therapy was discontinued if the platelet count was < 100,000/mm^3. The maximum cumulative dose of doxorubicin was 450 mg/m^2. If creatinine was between 1.2 and 1.5 mg/dl, the dose of cisplatin was reduced by 25%; 50% of the dose was given if creatinine was 1.5–1.9 mg/dl. Cisplatin was discontinued if creatinine was > 1.9 mg/dl.

For comparison, a control group of patients with lung cancer who were not given chemotherapy was also analyzed. In 1976, 113 patients with newly diagnosed NSLC presented at the Veterans Administration Hospital, Nashville, TN. Of these, 94 patients had resectable NSLC, and 65 had the characteristics of patients in this CAP study. Patients > 80 years old and those who survived < 6 weeks after diagnosis were excluded from the control group since similar exclusions were prospectively applied to the CAP group. Only one patient in the control group was a female. Otherwise the control group was comparable to the study group. Many of the patients in the study group were in the same Veterans Administration Hospital population from 1977 to 1978.

RESULTS

Response Rate and Survival

There were 54 evaluable patients in the study. PRs were seen in 14 of the 40 patients (35%) who had measurable lesions. Evaluable responses were seen in five of the 14 patients (36%) who did not have clearly measurable disease. Therefore, the response rate was similar for those with measurable disease and those with evaluable disease, giving an overall response rate of 35% (19 of 54 patients). Disease stability was seen in 17% of the patients, and 24% developed progressive disease during chemotherapy. Many of the patients whose disease did not regress by the second cycle (24%) requested that chemotherapy be discontinued and were classified as nonresponders (table 1).

The survival curves for the patients achieving PRs and for those with evaluable responses were identical, so these groups were combined. The survival curve for patients with stable disease was similar to the curve for those with disease progression, so these were combined (data not shown). The median survival for the 54 patients was 229 days, with the responders achieving a median of 380 days (range, 150–755+). In contrast, the nonresponders had a median survival of 150 days (range, 39–438) from the start of therapy (fig 1). Sixteen of the 35 nonresponders had died before the first responding patient died (150 days), and all of the nonresponders had died by 438 days, whereas eight of the 19 responders lived > 425 days. Two patients in this study are still alive at 726+ and 755+ days. Also shown in figure 1 is the survival curve for the 65 control patients who presented in 1976 and were not treated with chemotherapy. Patients with localized inoperable disease (39 of 65) received "curative" radiotherapy. Symptomatic care was given to all of the patients; palliative radiotherapy was given to 16 of the 65. Therefore, 55 of the 65 control patients received radiotherapy. The median survival was 165 days, with a range of 48–736 days. At 438 days, when all of the nonresponders to CAP had died, five of the 65 control patients were still living; they had locally advanced squamous cell lung cancer and were treated with curative radiotherapy. In contrast, at 438 days, eight of the 19 CAP responders were alive. The control group's overall survival curve approximates the nonresponder curve from the CAP study (fig 1).

In addition to the prolongation of median survival in the responders, there was general clinical improvement in these patients. Improvement of appetite, lessening of pain, decrease in hemoptysis and cough, and general improvement occurred in most of the responders. A determination of whether to continue chemotherapy could generally be made by the second or third cycle, when most of the responses were clinically evident.

Toxic effects from CAP were moderate. Bone marrow suppression was expected but was generally not severe. The nadir of leukopenia was gen-

TABLE 1.—Response to CAP therapy in patients with NSLC

	This study	Eagan 1977*	Eagan 1979†
No. of evaluable patients	54	41	42
Response rate, %‡	35	38	48
Adenocarcinoma	24 (4/17)	48 (6/14)	45 (10/22)
Squamous cell carcinoma	43 (9/21)	44 (8/18)	—
Large cell carcinoma	38 (6/16)	22 (2/9)	50 (10/20)
Median survival (days)			
Responders	380	393	340
Nonresponders	150	120	127
All patients	229	—	—
Prior therapy, %§			None
Radiation therapy only	24 (13/54)		
Radiation therapy		17 (7/41)	

*Ref 6.
†Ref 8.
‡Values in parentheses = No. of patients responding/No. of evaluable patients.
§Values in parentheses = No. of patients who had had prior radiation therapy/No. of evaluable patients.

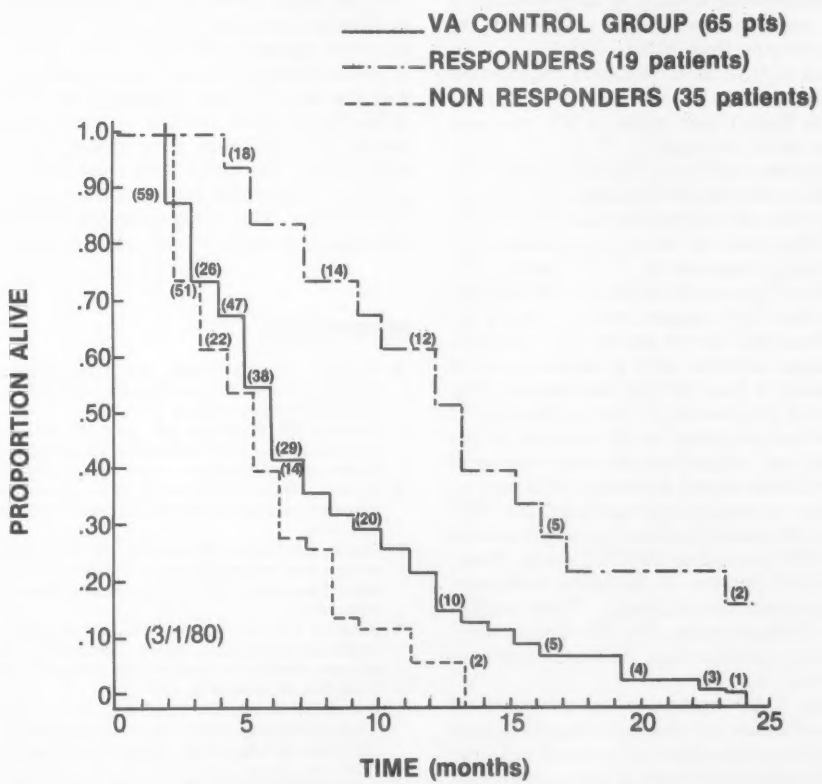

FIGURE 1.—Survival in CAP responders and nonresponders and in control patients.

erally > 1000 cells/mm^3, and there were no serious infections related to this relatively brief and mild leukopenia. There were no episodes of bleeding related to chemotherapy. Thirty percent of the patients (16 of 54) required transfusions for drug-induced anemia. A rise in serum creatinine of ≥ 0.3 mg/dl was noted in nine of the 54 patients (17%). No serious renal damage occurred in this study. Nausea and vomiting were universal and generally lasted 24–36 hours following drug administration. Cycle length was 4 weeks, and most patients recovered their appetites and ate normally for the final 2 weeks of each treatment cycle. Occasionally, patients had prolonged anorexia related to the chemotherapy, to the tumor, or to both. Eight patients stopped therapy voluntarily because of persistent vomiting.

DISCUSSION

Systemic chemotherapy for unresectable NSLC has had a disappointing history. A large number of combination regimens have been used, with response rates ranging from 10% to 40%.

Our interest in CAP as a treatment regimen for NSLC was prompted by the initial studies of Eagan et al (6) at the Mayo Clinic, where a 38% response rate was seen, with prolongation of survival in the responding patients. In followup studies, Eagan and co-workers (8) used a higher cisplatin dose (60 mg/m^2) and observed a 48% regression rate. Both Gralla et al (9) and Bjornsson et al (10) have found CAP useful in inducing responses in NSLC and in prolonging survival. Our results with these 54 patients treated with the CAP regimen indicate that a response rate from 35% to 40% can be expected with this combination regimen, with a median survival of approximately 1 year for the responders. This median survival represents at least a doubling of expected survival compared to the survival of the nonresponders and a significant increase compared to the survival of the group as a whole (385 days vs 229). We have retrospectively analyzed the data from a group of 65 patients presenting at our service in the year (1976) preceding the CAP study. These patients received curative or palliative radiotherapy and no systemic chemotherapy. Their median survival was 165 days (range, 48–736). The survival curve for this group approximates that of our nonresponder group (fig 1).

Although the CAP regimen in the treatment of NSLC does not induce the dramatic responses seen with combination chemotherapy in small cell lung cancer, approximately one third of the patients with NSLC do respond to treatment with this combination regimen. For those patients, there is generally symptomatic improvement, with a prolongation of median survival to approximately 1 year. Within this subgroup there are patients who have a very prolonged survival (> 2 years) after combination chemotherapy. No similar group was seen in the nonresponders, in the patients with stable disease, or in our historical controls who had been given radiotherapy only.

Based on the chemotherapeutic results to date, a nontreatment control group does not appear warranted for these neoplasms since it has been shown that chemotherapy can prolong survival for responding patients. Although occasional patients have been reported to survive for > 2 years without specific therapy for metastatic NSLC, in our series, longer survivals occurred only after a response to therapy. In our historical control group, a few patients (five of 65) lived longer than 438 days (the time when all nonresponders to CAP had died), and all had *locally advanced* unresectable squamous cell lung cancer. All received curative radiotherapy. In contrast, eight of the 19 CAP responders were alive at 438 days, and four of the eight had had clinically apparent distant metastases prior to the initiation of chemotherapy. Four of the 21 patients with limited disease who were treated with CAP and five of the 39 who were treated with radiotherapy (historical controls) were alive at 438 days. Patients with NSLC, like those with most other malignancies, live longer and better if the tumor responds to treatment. This study indicates that CAP chemotherapy is useful for some patients with NSLC.

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Cyclophosphamide, Doxorubicin, and Cisplatin in the Treatment of Non-Small Cell Bronchogenic Carcinoma [1,2]

W. K. Evans,* R. Feld, G. DeBoer, D. Osoba, J. E. Curtis, M. A. Baker, R. E. Myers, I. C. Quirt, K. I. Pritchard, T. C. Brown, G. J. Kutas, M. E. Blackstein, B. Ottema, and L. Millband [3,4]

SUMMARY

One hundred and forty-three patients with unresectable non-small cell bronchogenic carcinoma were treated with combination chemotherapy consisting of cyclophosphamide, doxorubicin, and cisplatin (CAP). Objective responses were seen in 27.5% of 131 evaluable patients. Response rates for squamous cell carcinoma, adenocarcinoma, and large cell anaplastic carcinoma were 30.2% (13 of 43 patients), 28.0% (14 of 50), and 32.1% (nine of 28), respectively. The median survival time for responders with extensive disease was 33.0 weeks compared with 29.3 weeks for patients with stable disease and only 9.6 weeks for patients with disease progression. The survival advantage of patients responding to CAP relative to those who had disease progression during treatment is highly significant statistically ($P = 0.0005$). However, patients whose disease remained stable also had longer survival than those who had disease progression ($P = 0.001$), and their survival was not significantly different from that of responders ($P = 0.19$). The CAP chemotherapy regimen was generally well-tolerated, although acute gastrointestinal symptoms were common. Our results indicate that CAP chemotherapy can cause tumor regression in patients with non-small cell bronchogenic carcinoma and may extend the survival of responding patients.

[Cancer Treat Rep 65:947–954, 1981]

Although a number of chemotherapeutic agents are reported to cause tumor regression in 15%–30% of patients with inoperable non-small cell bronchogenic carcinoma (NSCBC), none has demonstrated a major impact on survival (1). Furthermore, most combination chemotherapy regimens have only succeeded in increasing morbidity without meaningfully extending survival (2). Among the more active agents against NSCBC are doxorubicin and cyclophosphamide. In combination, these agents act synergistically in some animal tumor systems (3). This combination has demonstrated limited activity in several small phase II trials (4–7), and in a Veterans Administration group study, it extended the median survival time of patients with extensive squamous cell carcinoma beyond that achieved with cyclophosphamide alone or with several other drug combinations (8).

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[3]The Ontario Cancer Institute incorporating The Princess Margaret Hospital (*R. Feld, G. DeBoer, J. E. Curtis, I. C. Quirt, T. C. Brown,* and *B. Ottema*), Toronto General Hospital (*W. K. Evans* and *L. Millband*), Sunnybrook Medical Center (*D. Osoba*), Toronto Western Hospital (*M. A. Baker*), St. Michael's Hospital (*R. E. Myers*), Women's College Hospital (*K. I. Pritchard* and *G. J. Kutas*), and Mount Sinai Hospital (*M. E. Blackstein*), University of Toronto, Canada.

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**Reprint requests to*: William K. Evans, MD, Division of Hematology-Oncology, Toronto General Hospital, 101 College St, Toronto, Ontario M5G 1L7, Canada.

Although cisplatin has shown only minimal activity against NSCBC in phase II testing, Eagan et al (9) added this agent to the combination of cyclophosphamide and doxorubicin (CAP-1) because of evidence of synergism in some animal systems (10,11) and reported a 39% response rate in previously untreated patients with extensive disease. In this study, we have confirmed that CAP has activity against NSCBC and have observed that response rates are similar for the major histologic cell types.

PATIENTS AND METHODS

One hundred and forty-three patients with NSCBC were entered in the study between June 1977 and November 1979. All patients had either histologic or cytologic proof of NSCBC. Disease extent was defined by both clinical assessment and radiographic and radioisotope studies including bone, liver, and brain scans. Limited disease was defined as disease confined to a hemithorax and ipsilateral supraclavicular fossa. Extensive disease was defined as more distant tumor dissemination. Patients who had had prior chemotherapy were excluded from the study, but patients previously treated by surgery or radiotherapy were acceptable if there was evidence of disease progression in the radiation field or new extrathoracic disease. Radiation was also used to treat documented brain metastases and symptomatic bone lesions.

Patients were excluded from the study if the serum creatinine was > 1.5 mg/dl, if life expectancy was < 4 weeks, or if there was a history of myocardial infarction within the previous 3 months or a cardiac arrhythmia requiring anti-arrhythmic medication. A maximum of 12 courses of CAP chemotherapy was recommended to limit the cumulative dose of doxorubicin to 480 mg/m^2. The dose of cisplatin was adjusted for creatinine levels < 2.0 mg and was permanently discontinued if the serum creatinine increased to ≥ 2.0 mg/dl. Drug dose adjustments for hematologic toxicity were made for cyclophosphamide and doxorubicin according to nadir and treatment day blood counts. Doxorubicin dose was also modified for hyperbilirubinemia, and no drug was given if the bilirubin exceeded 2.0 mg/dl. The Eastern Cooperative Oncology Group (ECOG) scale (0–4) was used to evaluate the performance status of patients prior to starting chemotherapy.

The combination chemotherapy consisted of cyclophosphamide (400 mg/m^2), doxorubicin (40 mg/m^2), and cisplatin (40 mg/m^2), and all three drugs were given by bolus injection into the tubing of a freely flowing iv infusion. Treatments were repeated at 4-week intervals. No specific precautions were taken to hydrate patients prior to or during cisplatin administration. The majority of patients were treated as outpatients after premedication with standard anti-emetics, most commonly, prochlorperazine.

Pretreatment blood tests consisted of hemoglobin, total and differential wbc counts, and glucose and electrolytes, as well as tests of liver and renal functions. Blood counts were measured 10–14 days after each treatment and prior to each subsequent treatment. Chest radiographs were repeated monthly while treatment was being given. Electrocardiograms were obtained initially and at 2-month intervals or as indicated by the clinical circumstances. Liver and bone scintiscans were performed whenever biochemical tests or clinical symptoms warranted and to document response if the scan was abnormal initially.

In this study, a complete response (CR) was defined as the total disappearance of all tumor and all biochemical abnormalities indicative of tumor for at least 4 weeks. A partial response (PR) was defined as a $\geq 50\%$ decrease in the product of the longest perpendicular diameters of measurable lesions, lasting > 4 weeks, without the appearance of new lesions or growth of other measured lesions; for disease that was evaluable but not measurable, an estimated decrease of 75% in tumor size, as assessed by two observers, was required. Stable disease was defined as a $< 50\%$ reduction in tumor size or no definite increase in tumor size for > 8 weeks. Progressive disease was defined as an increase of $> 25\%$ in tumor size. All response rates were compared using the Fisher exact test (two-tailed value). Survival times were calculated from Day 1 of CAP chemotherapy until death. Actuarial survival curves were prepared using the Kaplan and Meier method (12). Comparisons of survival were made using the two-tailed generalized Wilcoxon-Gehan test (13).

RESULTS

Patient Characteristics

Of the 143 patients admitted to the study, 12 were inevaluable for response. There were six early deaths (death within 2 weeks of starting chemotherapy), three patients refused further chemotherapy after a single course of CAP because of drug-induced toxic effects, and in three cases, response could not be assessed because the indicator lesion was not re-evaluated by appropriate re-staging procedures.

The principal characteristics of the 131 patients evaluable for response are shown in table 1. Most of the patients were male; the median age was 58 years. There were 34 patients with limited disease and 97 with extensive disease. Patients with limited

TABLE 1.—Characteristics of 131 patients evaluable for response to CAP*

Characteristic	Limited disease	Extensive disease	Total
Sex			
Male	28	69	97
Female	6	28	34
Age (yrs)			
Median	59	58	58
Range	28–81	20–84	20–84
ECOG performance status			
0	8	6	14
1	21	65	86
2	5	18	23
3	0	8	8

*Unless specified otherwise, values = No. of patients.

TABLE 2.—Previous treatment of patients evaluable for response to CAP*

Treatment	Limited disease	Extensive disease	Total
Surgery (no radiotherapy)	1	5	6
Talc poudrage	2	1	3
Radiotherapy (no surgery)	8	34	42
Surgery and radiotherapy	5	9	14
Total	16	49	65

*Values = No. of patients.

TABLE 3.—Responses of 131 patients to CAP chemotherapy*

Carcinoma cell type	CR (%)	PR (%)	No change	Disease progression	Total
Squamous	—	13 (30.2)	15	15	43
Adenocarcinoma	1 (1.8)	13 (26)	18	18	50
Large cell anaplastic	1 (3.6)	8 (28.6)	10	9	28
Bronchoalveolar	—	—	4	4	8
Adenosquamous	—	—	—	2	2
Total	2 (1.5)	34 (25.9)	47	48	131

*Unless specified otherwise, values = No. of patients.

disease generally had a better performance status than patients with extensive disease. Sixty-five patients had received prior treatment (table 2), including 56 who had had irradiation to the primary site.

Response and Survival

Thirty-six responses were observed during treatment of the 131 evaluable patients, for an overall response rate of 27.5% (table 3). The only two CRs were in a patient with limited adenocarcinoma (response duration, 32 weeks; survival, 59 weeks) and a patient with extensive large cell anaplastic carcinoma (response duration, 23 weeks; survival, 85 weeks). Stable disease was observed in 35.8% of the patients (47) and disease progression occurred in 36.7% (48).

There were 26 responses among the 97 evaluable patients with extensive disease (response rate, 26.8%); 43 patients with extensive disease had received previous radiation therapy to the site of the primary tumor. Although the response rate for the 54 previously untreated patients with extensive disease (29.6%) was better than that for the previously irradiated patients (23.3%), it was not significantly greater ($P = 0.3$). For patients with limited

disease, the response rate was only minimally better than that for the group with extensive disease (29.4%; ten of 34), despite the fact that patients with limited disease had somewhat better performance status.

When the response data for both limited and extensive disease are analyzed according to cell type, it is seen that CAP is equally active in all major histologic subtypes. The response rates for squamous cell carcinoma, adenocarcinoma, and large cell anaplastic carcinoma were 30.2% (13 of 43 patients), 28.0% (14 of 50), and 32.1% (nine of 28), respectively (table 3). No responses were seen in any of the ten patients with other histologic subtypes: bronchoalveolar (eight) and adenosquamous (two) carcinomas.

The median survival time for the entire group of 104 patients with extensive disease was 15.3 weeks. The median survival times for patients with extensive squamous cell carcinoma, adenocarcinoma, and large cell anaplastic carcinoma were not significantly different: 13.1, 14.1, and 18.5 weeks, respectively. For the 97 patients with extensive disease who were evaluable for response, the median survival time was 17.9 weeks, and the survival curves for this group by response to CAP are shown in figure 1. The median survival time of responders was 33.0 weeks compared with 29.3 weeks for patients with stable disease and only 9.6 weeks for patients with disease progression. The survival advantage of patients responding to CAP over those who had disease progression with treatment is highly significant statistically ($P = 0.0005$). Furthermore, there is a significant survival advantage for responders compared to the whole group of evaluable patients with extensive disease ($P = 0.002$). Patients whose disease remained stable with treatment also had significantly longer survival than patients with disease progression ($P = 0.001$), and their survival was not significantly different from that of responders ($P = 0.19$).

The dependence of survival of patients with extensive disease on ECOG performance status was investigated by the log-rank method (14). Figure 2 shows a very strong trend toward poor survival with deteriorating performance status; this trend

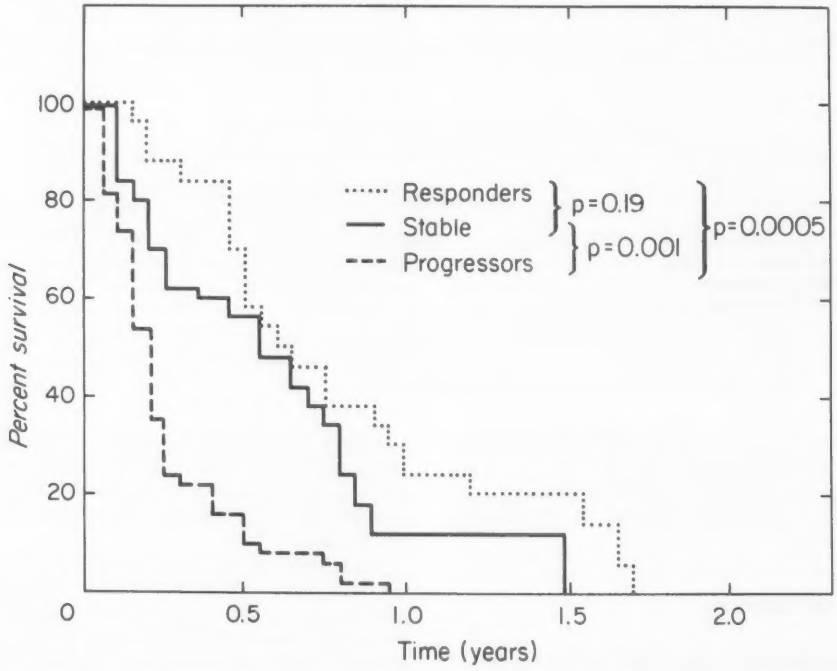

FIGURE 1.—Survival curves for patients with extensive disease, showing a comparison by response to treatment. There were 26 responses among 97 evaluable patients; Nos. of patients surviving at 6-mo intervals after time of entry into the study were 16 (6 mos), 7 (12 mos), and 3 (18 mos). 30 patients had stable disease; Nos. of patients surviving at 6-mo intervals were 15, 2, and 0. 41 had disease progression during CAP treatment; Nos. of patients surviving at 6-mo intervals were 4 and 0.

was highly significant statistically. In fact, the influence of performance status on survival was far greater than any effect of prior treatment on the outcome of treatment of patients with extensive disease.

Of the 39 patients with limited disease, 13 (33.3%) had been treated with radiotherapy prior to showing disease progression at the primary site. The majority of the patients with limited disease were treated with CAP until tumor progression was observed (one to thirteen cycles; mean, 3.5). However, three patients refused further treatment after a single course of CAP, and three received irradiation after the third course of CAP, in an effort to consolidate PRs and minimize the need for further chemotherapy. At the time of disease progression, eight previously non-irradiated patients received radiation (2500–3000 rads in ten to fifteen fractions) to the primary site. Thirty-four patients were evaluable for response to CAP, and the median survival time of this group was 41.9 weeks There were ten responders whose median survival time was 51.9 weeks compared with 34.7 weeks for the 24 nonresponders. This difference is not statistically significant ($P = 0.35$).

Toxicity

Alopecia, nausea, and vomiting were universal. In 17% of the patients, vomiting was severe, despite the use of standard anti-emetics. Three patients refused further treatment after a single course of CAP because of distressing gastrointestinal symptoms, even though dramatic tumor regression had been observed and the patients were encouraged to continue treatment. Three other patients refused treatment after two to four courses of CAP. Six patients developed chronic anorexia and progressively lost weight despite disease stability as shown radiologically.

Mild to moderate myelosuppression was common; in 36% of the patients, one or more courses of treatment were complicated by neutropenia of < 1000 granulocytes/mm^3, but only 12% had a platelet count

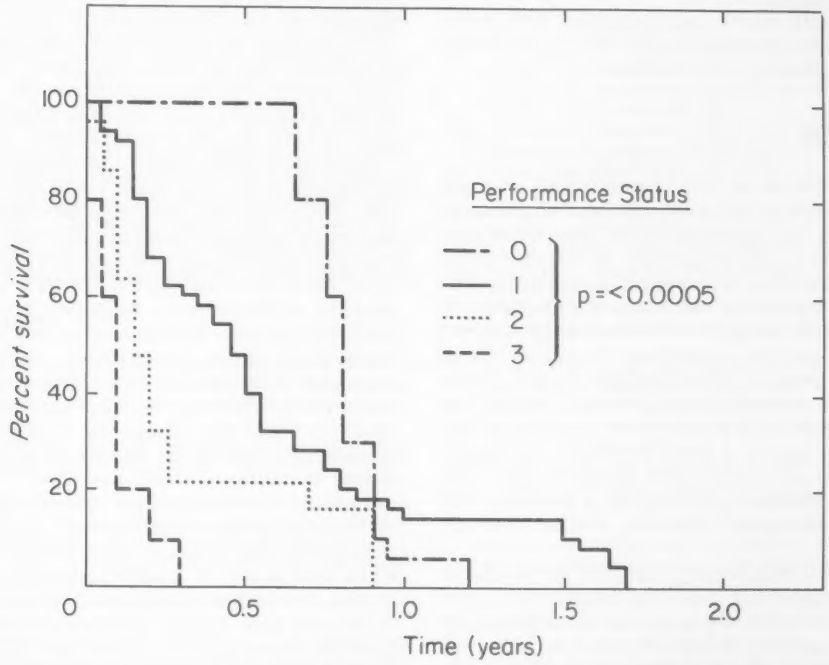

FIGURE 2.—Survival curves for patients with extensive disease, showing the dependence on ECOG performance status. No. of patients alive and under observation at the time of entry and at 6-mo intervals thereafter was as follows. Performance status of 0 (6, 5, and 0 patients); 1 (68, 27, 8, and 3); 2 (20, 4, 1, and 0); and 3 (10 and 0).

nadir ≤ 100,000 mm^3. There were no episodes of hemorrhage, but four infections were observed (three pneumonia and one superficial soft tissue), including one fatal pneumonitis. Two patients experienced culture-negative febrile episodes during periods of myelosuppression, requiring treatment with broad-spectrum antibiotics.

Nine of 52 patients (17.3%) who received two or more courses of CAP chemotherapy had increases in the serum creatinine above baseline levels following treatment but none exceeded 1.5 mg/dl. In two cases the serum creatinine reached its highest level (1.4 and 1.5 mg/dl) at the third course of treatment and then returned to normal despite another three to eight courses of CAP. In no case was azotemia a reason for interrupting CAP chemotherapy.

There was no clinical evidence of auditory toxic effects or peripheral neuropathy during this study. However, one patient exceeded the recommended total cumulative doxorubicin dose of 480 mg/m^2 because a stable PR had been achieved and was continuing. At a cumulative dose of 520 mg/m^2, he developed doxorubicin cardiotoxicity, documented by gated nuclear angiography and endomyocardial biopsy. Symptoms of congestive heart failure were controlled with digoxin, but the patient died of progressive tumor complicated by cerebral embolization from marantic endocarditis.

DISCUSSION

Despite efforts to find more effective systemic therapies for NSCBC, most studies of single-agent combination chemotherapy have failed to demonstrate useful antitumor activity. In a review of the literature in 1977, Livingston (2) concluded that, relative to supportive care alone, no combination of chemotherapeutic agents studied in large numbers of patients had had a significant impact on overall survival in patients with extensive disease. These studies have generally shown a median survival time of approximately 14 weeks, with < 10% of the patients surviving for 1 year. Therefore, the report of Eagan et al (11) of a 39% response rate, with significant extension of survival in patients with previously untreated extensive NSCBC, was encouraging.

The overall response rate in this study was 27.5%. The somewhat lower overall response rate may be due to the inclusion of a large number of previously irradiated patients, the use of somewhat more rigorous response criteria, and the inclusion of a group of patients with "other" non-small cell histologic subtypes that did not respond. For example, none of the eight patients with bronchoalveolar carcinoma responded to CAP, and although this is a small number of patients, bronchoalveolar carcinoma appears to be a particularly chemotherapy-resistant form of NSCBC (15). As demonstrated in other studies (11,16), performance status has a strong influence on the probability of response to chemotherapy. Twenty-six of the patients with extensive disease had a performance status of 2–3, and only three patients responded in this group (11.5%). On the other hand, 22 responses (30.9%) were observed in 71 patients with a good performance status (0–1). This difference approaches statistical significance ($P = 0.09$). In addition, performance status has a strong influence on survival, as shown in figure 2. The proportion of patients with extensive disease who had a good performance status (0–1) in this study (87%) was greater than in Eagan's (68%), and therefore, performance status distribution cannot explain the lower response rate observed.

In this study, the median survival time of the entire group of patients with extensive disease was 15.3 weeks, which is similar to that reported for supportive care alone. However, 26.9% of responding patients with extensive disease were alive at 1 year, and the median survival time of this group of patients was significantly greater than that of the whole group as well as that of patients with disease progression. On the other hand, only 7.2% of the entire group of evaluable patients with extensive disease survived 1 year, which is again similar to survival with supportive care alone.

Although moderate bone marrow toxicity was seen, myelosuppression was predictable and rarely severe. There was only one drug-related death in this series and important nephrotoxicity was not seen. The most troublesome problem with this regimen is its gastrointestinal toxicity, which is in large measure attributable to cisplatin. A significant number of patients experienced severe nausea and vomiting, which led some patients to refuse further treatment. The standard anti-emetics used in this study, prochlorperazine and dimenhydrinate, were relatively ineffective. Improved control of emesis by new anti-emetics is unlikely to influence the chronic anorexia and debilitation observed in some patients who receive multiple cycles of therapy.

The contribution of cisplatin to the antitumor activity of the CAP combination could be questioned. When used in low to moderate doses in six phase II trials, cisplatin produced only seven responses in 75 patients with NSCBC (9% response rate) (17). However, when high doses were used (120 mg/m^2), the Lung Cancer Working Party of the European Organization for Research on Treatment of Cancer observed a PR rate of 26% in 62 NSCBC patients (18). Efforts to improve the efficacy of CAP chem-

otherapy by using higher doses of cisplatin have produced conflicting results. Eagan et al (19) treated 42 patients with large cell anaplastic carcinoma and adenocarcinoma, using doses of 60 mg/m² of platinum as a 6-hour infusion. A 48% response rate was observed. On the other hand, Gralla et al (17), using even higher doses (120 mg/m²), achieved a 27.5% response rate in 46 evaluable patients, a response rate similar to that achieved in the present study. Although there are other drug combinations with activity against NSCBC, none of these appears to be more effective than CAP. Initial reports with cyclophosphamide, doxorubicin, methotrexate, and procarbazine (CAMP) (16,20) and methotrexate, doxorubicin, cyclophosphamide, and CCNU (MACC) (21) indicated better response rates than subsequent studies were able to achieve (22–24). Other encouraging reports that require confirmation include the use of 5-FU, doxorubicin, and mitomycin C (FAM) by Butler et al (25) in adenocarcinoma of the lung and the use of a CAP regimen with the addition of CCNU and vincristine by Takita et al (26).

CAP has now been evaluated in 348 patients by four groups of investigators (11,17,19,27). The response rates range from 27.5% to 48% and average 30%. Responders have had a statistically significant survival advantage over nonresponders in all studies. However, CAP is not sufficiently active in a large enough proportion of NSCBC patients to influence the median survival time of the group as a whole. Although this level of antitumor activity is far from optimal, it does appear that some progress has been made against NSCBC. Encouraged by these results, the Lung Cancer Study Group is now exploring the use of CAP as adjuvant therapy in NSCBC that is resectable but has a poor prognosis.

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Combination Cyclophosphamide, Doxorubicin, and Cisplatin (CAP) Chemotherapy for Extensive Non-Small Cell Carcinomas of the Lung[1]

Stephen Davis,[2,*] Pietro Rambotti,[2,3] and Young K. Park[2]

SUMMARY

Fifty consecutive patients with extensive non-small cell carcinoma of the lung were randomized to one of two chemotherapeutic regimens. Twenty-three patients (group 1) received cyclophosphamide (600 mg/m²), doxorubicin (40 mg/m²), and cisplatin (50 mg/m²) every 3 weeks; 27 patients (group 2) received cyclophosphamide (600 mg/m²), doxorubicin (40 mg/m²), and cisplatin (100 mg/m²) every 3 weeks. The objective response rates were 4% and 7% in groups 1 and 2, respectively. Median survival duration was 15.2 weeks (group 1) and 21.7 weeks (group 2; $P \geq 0.3$). Hematologic toxicity was minimal in group 1 and moderate in group 2. Renal toxicity was moderate in group 2 only. Combination chemotherapy with cyclophosphamide, doxorubicin, and cisplatin as used in this study is not superior to previous studies in this institution using cyclophosphamide as a single agent.

[Cancer Treat Rep 65:955–958, 1981]

Single-agent chemotherapy for patients with extensive non-small cell bronchogenic carcinoma has proved to be of limited therapeutic significance. In spite of initial enthusiasm, large studies reveal a response rate between 0 and 15% (1). Combination chemotherapy regimens have been reported to increase survival in this disease. Hyde et al (2) and Hansen et al (3) suggested that multimodal chemotherapy increased median survival in squamous cell carcinoma and adenocarcinoma, respectively. Larger studies have not conclusively substantiated this claim (4,5).

Cisplatin is a newly released platinum coordination complex with marked antitumor activity. It is believed to be synergistic with other chemotherapeutic agents in inducing a therapeutic effect in patients with non-small cell lung cancer. Takita et al (6) reported a 66% major response (complete and partial) rate using cisplatin, cyclophosphamide, doxorubicin, CCNU, and vincristine. Similarly, Eagan and co-workers (7) found that cyclophosphamide, doxorubicin, and cisplatin (CAP) induced a 42% response rate. In an attempt to evaluate the role of CAP in extensive non-small cell lung cancer, we performed a randomized study comparing two CAP regimens.

MATERIALS AND METHODS

From January 1978 to March 1979 a total of 64 patients with biopsy-proven non-small cell carcinoma of the lung were entered in the study; only 50 were evaluable. There was no selection but patients were required to have a Karnofsky performance status (PS) > 40 and to have extensive disease (tumor not confined to mediastinum and one hemithorax). Patients had clinically detectable disease measurable either by physical examination of mass or by roentgenographic techniques. The group consisted of 46 men and four women who were 47–78 years of age (mean, 61). No patient entering the protocol had received prior chemotherapy but most

[1]Received Aug 21, 1980; revised Dec 5, 1980; accepted Dec 18, 1980.

[2]Oncology/Clinical Hematology Section, Veterans Administration Medical Center, East Orange, NJ, and the CMDNJ-New Jersey Medical School, Newark.

[3]On leave from the Istituto di Clinica Medica Generale e Terapia Medica, Università di Perugia, Italy.

**Reprint requests to:* Stephen Davis, MD, Department of Oncology/Clinical Hematology, Veterans Administration Medical Center, East Orange, NJ 07019.

TABLE 1.—Patient characteristics*

Characteristic		Group 1	Group 2
Age (yrs)			
Mean		58	63
Range		50–74	47–78
Evaluable patients		23	27
Histologic diagnosis†			
Squamous cell carcinoma	11	3	3
	12	1	2
	13	2	1
Adenocarcinoma	31	4	6
	32	4	6
	33	3	5
Large cell carcinoma	40	6	4
Prior therapy			
Surgery		2	4
Radiotherapy		17	21
PS			
40–70		17	22
80–100		6	5
Weight loss‡		14	16

*Unless specified otherwise, values = No. of patients.
†World Health Organization (1977) classification.
‡No. of patients presenting with > 10% loss of body weight in the 6-mo period prior to diagnosis.

(76%) had received prior radiotherapy. Patient characteristics are shown in table 1.

Pretreatment evaluation consisted of a history and physical examination, blood cell count, liver function tests, BUN, creatinine, urinalysis, chest roentgenogram, radionuclide scanning of liver and bone, and gallium-67 total-body scanning.

Patients were evaluated every 3 weeks by routine studies. Radionuclide scanning was repeated as indicated clinically. A partial response was defined as a > 50% decrease in the total area of all measurable disease, with no evidence of new malignant disease. Patients who had a subjective response, ie, decrease in pain but no associated decrease in measurable tumor burden as defined above, were considered nonresponders. Disease "stabilization" was not used in the evaluation. Survival calculations for patients entering this study were evaluated by the two-tailed generalized Wilcoxon-Gehan test (8).

Patients were randomized to receive the following courses of chemotherapy: group 1 patients received cyclophosphamide (600 mg/m² iv), doxorubicin (40 mg/m²), and cisplatin (50 mg/m²) every 3 weeks. Group 2 patients received cyclophosphamide (600 mg/m² iv), doxorubicin (40 mg/m²), and cisplatin (100 mg/m²) every 3 weeks. Cisplatin, at this dose, was given with mannitol diuresis (9). Chemotherapy was continued until no further decrease in measurable disease occurred or obvious disease progression could be documented. Patients were considered to have had an adequate trial if they were in the study for at least 21 days. Planned drug administration was delayed if the wbc count was < 3000/μl or the platelet count was < 75,000/μl. Patients with such transient leukopenia or thrombocytopenia were then followed weekly and treated as soon as counts increased above these levels.

RESULTS

Therapeutic Benefits

Two to eight courses of therapy (mean, 3.4) were given to the cohort of 50 evaluable patients. The objective response rates were 4% and 7% in groups 1 and 2, respectively. There were no complete responses. The three patients who had partial responses had PS values of 80, 60, and 60. In group 1 the only response was in a patient with squamous cell carcinoma (WHO 11); in group 2 both responders had well-differentiated adenocarcinoma (WHO 31).

The survival rates for all patients in each group are shown in figure 1. The median survival in group 1 was 15.2 weeks and in group 2 was 21.7 weeks ($P \geq 0.3$). Survival in the three partial responders was 31.7 weeks. As can be seen in figure 1, median survival for both groups is similar to that of patients at this institution who were treated with cyclophosphamide alone (10). With regard to prognostic variables, the patients in this and our previous study are similar.

Toxic Effects

The toxic effects of the chemotherapeutic protocols are summarized in table 2. The degree of neutropenia and thrombocytopenia was classified as moderate in group 2 but minimal in group 1. There were no drug-related deaths. Infection, documented by positive microbiologic cultures, was seen in three patients (11%) in group 2. Renal dysfunction was seen in 11 patients (40%) in group 2 and two patients (4%) in group 1. This was manifested as an increase in serum creatinine levels ≥ 2 mg/dl above baseline or as acute electrolyte losses, ie, hypokalemia, hypomagnesium, hypocalcemia, and hypophosphatemia. These renal disturbances are due to the effect of cisplatin on renal tubules. We have previously reported in greater detail on these renal abnormalities (9).

DISCUSSION

This study evaluated the efficacy of CAP combination chemotherapy in the treatment of exten-

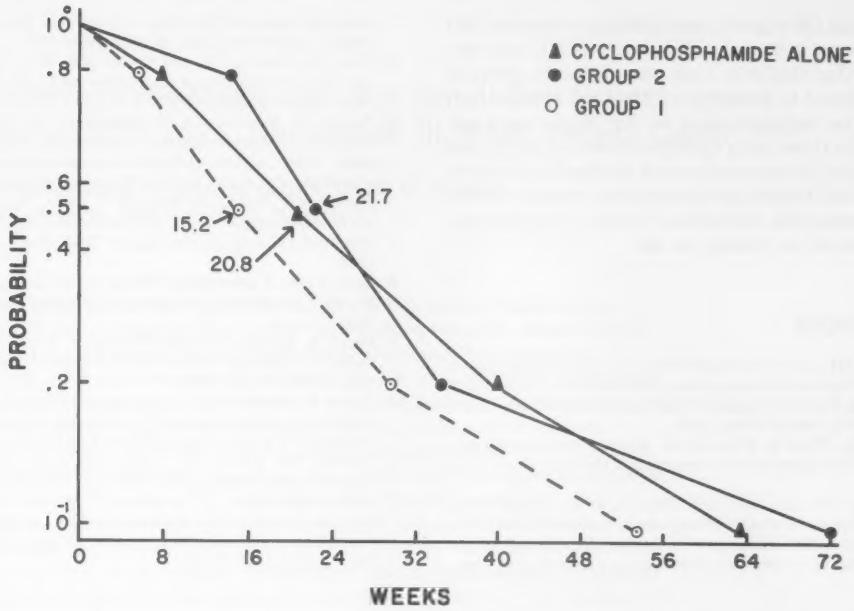

FIGURE 1.—Survival after chemotherapy for extensive non-small cell carcinoma of the lung. Indicated median survival times are from start of treatment. Data on patients given cyclophosphamide alone (600 mg/m² every 3 wks) have been published (10).

TABLE 2.—Toxic effects with CAP

	No. of patients	
	Group 1	Group 2
Wbc count (cells/μl)		
< 1000	0	2
1000–2000	4	4
2000–3000	6	14
> 3000	13	7
Platelet count (cells/μl)		
< 20,000	0	1
20,000–50,000	8	15
> 50,000	15	11
Infection*	0	3
Renal†	2	11

*Culture-positive blood, sputum, urine, or spinal fluid.
†Serum creatinine elevations ≥ 2 mg/dl and/or electrolyte disturbances.

sive non-small cell carcinoma. The results indicated a response rate of 4% with 50 mg/m² of cisplatin and 7% with 100 mg/m² of cisplatin in group 1 and 2 regimens, respectively. When compared to single-agent chemotherapy with cyclophosphamide at this institution, no survival advantage could be seen with CAP protocols. These data vary from those of Eagan et al (7) and Britell et al (11) who both reported a 42% response and Takita et al (6) who reported a 66% response with CAP-containing regimens. It must be stressed, however, that these studies involved patients with limited disease and that the patients in our study represent, as a whole, a poorer prognostic group, ie, mean PS was 60, with 55% of the patients having a history of weight loss. Median survival, taking into account these most important of prognostic variables, must be less in our population since survival in non-small cell lung cancer is independent of chemotherapy. Larger Veterans Administration Lung Cancer Study Group investigations (2) have yielded a median survival similar to that in the present study.

Britell et al (11) and Eagan et al (7) used platinum-based combination chemotherapy in predominantly untreated patients, unlike our study in which 76% of the patients had been irradiated. It is not likely that this could account for the differences in drug efficacy. Takita et al (6) do not mention previous therapy in their publication. Britell (11) used cisplatin at doses of 40 mg/m² every 4 weeks along with cyclophosphamide (400 mg/m²) and doxorubicin (50 mg/m²); Takita (6) used cisplatin (50 mg/m²),

doxorubicin (50 mg/m^2), and cyclophosphamide (300 mg/m^2) every 4–6 weeks along with CCNU and vincristine. Our cisplatin dose was higher in group 2 but still failed to induce a survival advantage.

Since the regimens used in this study were not superior to those with cyclophosphamide alone, we suggest that future randomized studies in extensive non-small cell bronchogenic carcinoma consider newer chemotherapeutic modalities versus cyclophosphamide alone or no therapy at all.

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Chemotherapy for Metastatic Non-Small Cell Bronchogenic Carcinoma: EST 2575, Generation III, HAM Versus CAMP[1,2]

John C. Ruckdeschel,* Cyrus R. Mehta, Omar M. Salazar, Richard H. Creech, and Robert W. Sponzo[2,3]

SUMMARY

Between September 1978 and March 1979 the Eastern Cooperative Oncology Group compared the CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) and HAM (hexamethylmelamine, doxorubicin, and methotrexate) regimens in 154 patients with metastatic non-small cell bronchogenic carcinoma. Most patients were ambulatory (77%) and had not received prior radiotherapy (59%). HAM produced two complete responses (CR) and eight partial responses (PR) (n = 77; 2.5% CR, 10.4% PR, 13% overall response) whereas CAMP resulted in five CR and 12 PR (n = 77; 6.5% CR, 15.6% PR, 22% overall response). This difference was not statistically significant (P = 0.14 with Fisher's exact 2-sided test) nor was the difference in overall median survival (HAM, 22.1 weeks and CAMP, 19.3 weeks). Responders had a significantly improved median survival (32.5 weeks) compared to nonresponders (17.9 weeks, P = 0.018). Ambulatory performance status and lack of prior radiotherapy were positive predictors for prolonged survival.

[Cancer Treat Rep 65:959–963, 1981]

EST 2575 is a prospective, randomized clinical trial of the Eastern Cooperative Oncology Group (ECOG) that serves to screen for activity of new regimens for advanced non-small cell bronchogenic carcinoma and to evaluate groupwide efficacy of treatments reported to demonstrate activity in single-institution trials. Generation I demonstrated that the combination of hexamethylmelamine, doxorubicin, and methotrexate (HAM) produced a significantly improved response rate (27%) and median

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*Reprint requests to: John C. Ruckdeschel, MD, Division of Oncology, Department of Medicine, The Albany Medical College of Union University, Albany, NY 12208.

TABLE 1.—Dose schedules for the HAM and CAMP regimens

Regimen	Compound	Dose	Schedule and route
HAM	Hexamethylmelamine	100 mg	Daily, orally, continuous
	Doxorubicin	40 mg/m^2	Day 1, q3wks, iv
	Methotrexate	40 mg/m^2	Day 1, q3wks, iv
CAMP	Cyclophosphamide	300 mg/m^2	Days 1 and 8, q4wks, iv
	Doxorubicin	20 mg/m^2	Days 1 and 8, q4wks, iv
	Methotrexate	15 mg/m^2	Days 1 and 8, q4wks, iv
	Procarbazine	100 mg/m^2	Days 1–10, orally

survival duration (33.9 weeks) in patients with inoperable (locally advanced and metastatic) large cell anaplastic carcinoma and adenocarcinoma when compared to the contemporary ECOG "standard" treatment of cyclophosphamide plus CCNU (1). The current generation (III) sought to compare these results for HAM to the 35% response rate reported for CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) by Bitran et al (2). The results of this comparison are detailed in this report.

PATIENTS, MATERIALS, AND METHODS

Patients

Patients were required to have histologically confirmed, metastatic non-small cell bronchogenic carcinoma, either squamous cell carcinoma, large cell anaplastic carcinoma, or adenocarcinoma. Patients with disease confined to the ipsilateral lung and draining nodes were eligible for a separate study (EST 3578) and were excluded from this one, as were patients with an ECOG performance status ≥ 3, prior chemotherapy, prior radiation therapy to the only measurable disease, CNS metastases, or abnormal pretreatment laboratory values (creatinine > 1.5 mg/100 ml, bilirubin > 2.0 mg/100 ml, wbc count $< 4000/mm^3$ or platelet count $< 100,000/mm^3$). All patients gave informed written consent to participate in this clinical trial.

Study Design and Treatments

Patients were stratified by histologic cell type and initial performance status (0 or 1 vs 2). The doses and schedules for the two treatment regimens are listed in table 1. Doses were modified on the basis of wbc and platelet counts on the day of treatment and at the midcycle nadir, as described previously (3). Dose adjustments were also made for hepatic or renal dysfunction, severe nausea and vomiting, severe mucositis or cystitis, and marked CNS toxicity. Our response criteria are detailed elsewhere and consist only of complete response (CR) (the disappearance of all tumors) or partial response (PR) (a $> 50\%$ reduction in the product of the longest perpendicular diameters of the most clearly measurable lesion without progression of other lesions or a deterioration in weight or performance status) (1). Responses less than partial ($< 50\%$) were classified as "no change" and were listed with objective disease progression. Although this results in a somewhat more conservative estimation of response rates, it is in keeping with the most recent World Health Organization guidelines for the definition of solid tumor responses (4). Since primary lung cancer is frequently not measurable in two dimensions (eg, mediastinal masses), an 80% regression agreed on by two observers was required for a PR of such lesions.

RESULTS

Patient Characteristics

Of 173 patients initially entered from September 1978 to March 1979, 154 were eligible for analysis, with 77 on each regimen. Of the 19 ineligible patients, nine cancelled prior to therapy, four were lost to followup, three had a performance status ≥ 3, one had abnormal renal function, one had disease that was not histologically confirmed, and one had a lymphoma rather than lung cancer. Table 2 lists prognostic factors found to be of importance in earlier generations of EST 2575 (1,5) and shows their distribution by treatment. Treatment imbalances for prior radiotherapy and prior surgery were noted and adjusted for in the survival analysis.

Response

All 154 patients are included in the response analysis. The objective response rates for the two treatments, by cell type, are shown in table 3. The overall response rate for CAMP (22%) was not significantly different than that for HAM (13%) ($P = 0.14$ with Fisher's exact 2-sided test). The median time to response was 4.6 weeks for the 27 re-

TABLE 2.—Distribution of eligible patients by treatment and selected prognostic factors

Prognostic factor	Level of factor	No. of patients (%) treated with— HAM	CAMP
Cell type	Squamous cell carcinoma	32 (42)	30 (39)
	Adenocarcinoma	30 (39)	33 (43)
	Large cell anaplastic carcinoma	15 (19)	14 (18)
Initial performance status	0	13 (17)	13 (17)
	1	48 (62)	45 (58)
	2	16 (21)	19 (25)
Sex	Male	53 (69)	52 (67)
	Female	24 (31)	25 (33)
Prior wt loss	None	31 (40)	39 (51)
	<5%	18 (23)	12 (16)
	5%–10%	15 (20)	14 (18)
	> 10%	12 (16)	11 (14)
	Unknown	1 (1)	1 (1)
Prior radiotherapy	Yes	24 (31)	39 (51)
	No	53 (69)	38 (49)
Prior surgery	Yes	28 (36)	37 (48)
	No	49 (64)	40 (52)
Age	< 50 yrs	10 (13)	15 (19)
	50–60 yrs	20 (26)	24 (31)
	60–70 yrs	37 (48)	25 (33)
	> 70 yrs	6 (8)	8 (10)
	Unknown	4 (5)	5 (7)
Time from onset of symptoms	≤ 6 mos	46 (60)	46 (60)
	> 6 mos	27 (35)	25 (32)
	Unknown	4 (5)	6 (8)

TABLE 3.—Objective tumor response by treatment and cell type

	Squamous cell carcinoma		Adenocarcinoma		Large cell anaplastic carcinoma	
	HAM	CAMP	HAM	CAMP	HAM	CAMP
No. of patients with—						
CR	2	3	0	1	0	1
PR	4	6	4	5	0	1
No change	6	5	5	10	1	2
Disease progression	20	16	21	17	14	10
No. of patients treated	32	30	30	33	15	14
No. of responders	6	9	4	6	0	2
Response rate, CR + PR (%)	19	30	13	18	0	14

sponders, and the median duration of response was 11.5 weeks. Three patients survived > 70 weeks and there were a total of seven CRs (five with CAMP and two with HAM, 4.5% overall). Tumor responses were not significantly affected by the various prognostic factors (table 4), with the possible exception of prior surgery which conferred a marginal improvement ($P = 0.058$ with Fisher's exact 2-sided test). As noted in earlier generations of EST 2575, there were no differences in response by histologic subtype.

Survival

All 154 eligible patients are included in the survival analysis. Survival time was measured from the date of randomization to the date of death or to the date the patient was last known to be alive in

TABLE 4.—Objective tumor response and survival by selected prognostic factors

Prognostic factor	Level of factor	No. of patients treated	No. of responders	Response rate CR + PR (%)	Median survival (wks)
Performance status	0	26	6	23	26.9
	1	93	15	16	21.9
	2	35	6	17	15.2
Sex	Male	105	16	15	19.8
	Female	49	11	22	22.3
Prior wt loss*	None	70	13	19	22.4
	< 5%	30	4	13	19.9
	5%–10%	29	3	10	19.5
	> 10%	23	6	26	16.5
Prior radiotherapy	Yes	63	12	19	16.6
	No	91	15	16	25.9
Prior surgery	Yes	65	16	25	18.4
	No	89	11	12	22.0
Age*	< 50 yrs	25	3	12	14.2
	50–60 yrs	44	7	16	21.9
	60–70 yrs	62	10	16	21.9
	> 70 yrs	14	3	21	34.0
Time from onset of symptoms	≤ 6 mos	92	16	17	19.0
	> 6 mos	52	8	15	20.3

*Patients with unknown level of factor are omitted.

TABLE 5.—Predicted median survival by treatment and cell type*

Cell type	Median survival (wks)	
	HAM	CAMP
Squamous cell carcinoma	22.1	20.2
Adenocarcinoma	22.6	21.6
Large cell anaplastic carcinoma	17.5	16.6
Overall	21.6	20.0

*Predicted median survival times were adjusted for the prognostic effects of prior radiotherapy and initial performance status as described (6).

the case of censored observations. The effect of the various prognostic factors on survival is noted in table 4. Ambulatory performance status (ECOG 0 or 1), no prior radiotherapy, and age > 60 yrs have a significantly favorable effect on survival ($P = 0.001$, $P = 0.005$, and $P = 0.019$, respectively, using the log-rank test). Predicted median survival times by treatment and cell type are shown in table 5. These predictions were made by the Cox proportional hazards model (6) with appropriate adjustments for the prognostic effects of prior radiotherapy and initial performance status. There is no significant difference in survival between the treatments. The estimated median survival times are 32.6 weeks for responders and 17.9 weeks for nonresponders ($P = 0.018$, log-rank test).

Toxicity

As noted previously (5), complications are routinely monitored on a 6-point scale ranging from 0 (none) to 5 (drug-related death). All 154 patients were evaluable for toxic reactions. Table 6 lists the combined number of severe, life-threatening, and fatal toxic effects for each treatment. The two treatments are comparable with respect to toxicity. Most of the severe toxic effects and four of five drug deaths were related to hematologic toxicity.

TABLE 6.—Analysis of toxicity by treatment

Type of toxicity	No. of patients with severe, life-threatening, or fatal toxic reactions*	
	HAM ($n = 77$)	CAMP ($n = 77$)
Gastrointestinal (other than vomiting)	1	2
Vomiting	10	5
Infection	1	6 (1)
Bleeding	1	1
Skin or mucous membrane	1	0
Neurologic	0	0
Respiratory	1	0
Genitourinary	0	0
Hematologic	15 (4)	23
Cardiac	0	1

*Nos. in parentheses = No. of deaths.

DISCUSSION

When this study was designed, the most effective combination chemotherapy known for advanced non-small cell bronchogenic carcinoma was the CAMP regimen reported by Bitran et al (2) at the University of Chicago. They studied 61 patients with metastatic lung cancer, reporting a 35% response rate and an overall estimated median survival of 34 weeks (54 weeks for responders). The ECOG experience in testing drug regimens with reported efficacy in advanced lung cancer (generation II) suggested that these results should be confirmed in a groupwide trial (5). At that time, our most effective therapy was the HAM combination, which demonstrated a 27% response rate and a median survival of 33.9 weeks in patients with large cell anaplastic carcinoma and adenocarcinoma (1). In generation III, we compared these regimens in all three cell types since our previous experience suggested no difference in response by histologic subtype (1,5). Generation III differed from generation I in that the limitation to patients with metastatic disease resulted in a group with a poorer prognosis that was only partially compensated for by excluding patients with performance status 3.

The drop in overall response rate for HAM from 27% in generation I to 13% in generation III was not entirely unexpected. Generation III excluded patients with limited disease; five of 12 (42%) patients with limited large cell anaplastic carcinoma and adenocarcinoma responded to HAM in generation I whereas only six of 29 (21%) with extensive disease did so. Although all three cell types were included in generation III, HAM did better in the squamous cell subgroup (19% response) than it did in either the adenocarcinoma (13%) or large cell anaplastic subgroup (0). Generation III also employed a dose modification for nadir blood cell counts, which may have somewhat reduced the amount of drugs given although the effect would have been equivalent for CAMP. Although HAM has activity in advanced lung cancer, it appears no better than CAMP.

CAMP demonstrated an overall response rate of 22% (6.5% CR and 15.6% PR). This is less than the 35% response rate reported by Bitran et al (2), but the lesser rate could be accounted for by the somewhat more stringent ECOG response criteria used in this report and by the fact that the population studied at a single center is more uniformly screened (by process of referral) than that studied by a cooperative group. Bitran excluded ten patients from analysis; seven who did not complete one course of therapy and three who had irradiated brain metastases as the sole site of metastatic disease (2). This would drop the overall response rate to 29.5% (18 of 61 patients). It is difficult to compare their results directly because 11 patients had brain metastases and 16 received concurrent chest irradiation for local complications (eg, superior vena cava syndrome or obstructive pneumonitis). We feel that the practicing oncologist can reasonably expect a response rate on the order of 22% in this group of patients. The demonstration of five CRs (6.5%) in patients with metastatic non-small cell lung cancer is modestly encouraging since there is a survival advantage for responders. Although not significantly better than HAM ($P = 0.14$), CAMP is the most effective regimen tested by the ECOG to date in patients with metastatic non-small cell bronchogenic carcinoma.

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Chemotherapy for Inoperable, Non-Small Cell Bronchogenic Carcinoma: EST 2575, Generation II[1,2,3]

John C. Ruckdeschel,* Cyrus R. Mehta, Omar M. Salazar, Martin Cohen, Steven Vogl, Lawrence S. Koons, and Harvey Lerner[2,4]

SUMMARY

Between 1976 and 1978 the Eastern Cooperative Oncology Group tested ten regimens in 415 patients with histologically documented, inoperable non-small cell bronchogenic carcinoma. Most patients were ambulatory (69%) and had extensive disease (69%). Patients were stratified by cell type: squamous cell carcinoma (SQ), large cell anaplastic carcinoma (LC), or adenocarcinoma (AD). Ineffective single agents (including cell types tested and percent complete and partial responses) were dactinomycin (SQ, 6%), dianhydrogalactitol (SQ, 0), ftorafur (AD and LC, 3%), and piperazinedione (AD and LC, 7%). Ineffective combination regimens included the contemporary standard regimen cyclophosphamide (CYT) plus CCNU (SQ, AD, and LC, 9%), methotrexate plus doxorubicin (ADR) plus CYT plus CCNU (MACC) (SQ and AD, 12%), and mitolactol plus ADR (AD and LC, 8%). When compared to CYT plus CCNU the following regimens demonstrated significant activity: CYT plus bleomycin plus cisplatin (SQ, 23%; $P = 0.02$) and ADR plus 5-FU plus cisplatin (AD and LC, 24%; $P = 0.006$). Mitomycin demonstrated marginal activity in squamous cell cancer (19%, $P = 0.06$). Neither "active" regimen improved survival although responders to any regimen had a significant prolongation of median survival (31.6 vs 15.7 weeks, $P = 0.002$). The MACC regimen, piperazinedione, and mitomycin were substantially more toxic than the two effective

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**Reprint requests to*: John C. Ruckdeschel, MD, Division of Oncology, Department of Medicine, The Albany Medical College of Union University, Albany, NY 12208.

regimens, which were adequately tolerated. Ambulatory performance status, limited disease, and prior surgery were significant positive prognostic variables whereas prior radiation and pretreatment weight loss adversely affected response or survival.

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Although advances have been made in the treatment of small cell anaplastic lung cancer (1,2), the non-small cell subtypes remain both more common and more refractory to therapy (3). Single agents (4) and combinations of drugs (3) have been reported to demonstrate activity, but most lack confirmation in a multi-institutional trial.

EST 2575 is a prospective, randomized clinical trial of the Eastern Cooperative Oncology Group (ECOG) that serves to screen for activity of new regimens and to evaluate groupwide efficacy of treatments reported to show improved response rates in single institution trials. In the first generation of EST 2575, 284 eligible patients received seven different regimens, with the combination of hexamethylmelamine, doxorubicin, and methotrexate (HAM) causing a significantly improved response rate and survival when compared to the contemporary "standard" treatment of cyclophosphamide (CYT) and CCNU (5). Generation II began accruing patients in October 1976 during the followup period for generation I, and 415 eligible patients were treated through June 1978. Nine new regimens were compared to CYT plus CCNU, and the results serve as the basis for this report.

PATIENTS, MATERIALS, AND METHODS

Patients

Patients were required to have histologically documented, surgically incurable non-small cell bronchogenic carcinoma, either squamous cell carcinoma (SQ), large cell anaplastic carcinoma (LC), or adenocarcinoma (AD). Measurable or evaluable lesions were required and patients with either locally advanced or metastatic disease were accepted for the study. Patients with small cell anaplastic carcinoma were excluded as were patients who had an ECOG performance status of 4 (bedridden). Prior chemotherapy and irradiation to areas of measurable disease were further criteria for ineligibility. Adequate pretreatment renal (creatinine < 1.5 mg/100 ml), hepatic (bilirubin < 2.0 mg/100 ml), and marrow (wbc count $> 4000/mm^3$; platelet count $> 100,000/mm^3$) functions were required.

All patients gave informed written consent.

Study Design and Treatments

Patients were stratified by histologic subtype, performance status, extent of disease, sex, and institutional group. Two institutional groups were used to limit the number of drug regimens to three per institution for each cell type. These groups were chosen arbitrarily on an alphabetic basis with the goal of entering approximately equal numbers of patients in the two groups. The dose schedules for the individual regimens are listed in table 1. Not all regimens were tested in all three cell types. Doses were modified on the basis of wbc and platelet counts on the day of therapy and when hepatic or renal function was impaired. As described elsewhere (5), responses were defined as complete (CR) (the disappearance of all tumor) or partial (PR) (a $\geq 50\%$ reduction in the product of the longest perpendicular diameters without progression of other lesions or a deterioration in weight or performance status). Measurement of response in many "evaluable" pulmonary and mediastinal lesions is difficult (6), so that a PR required an estimated 80% regression agreed on by two investigators. Responses less than partial were listed as no change and were classified with objective disease progression. ECOG does not use a "minimal" response category, and hence response rates are conservatively estimated.

RESULTS

Patient Characteristics

Of 474 patients initially entered in the study, 415 were eligible for analysis. Of the 59 ineligible patients, 22 cancelled prior to therapy, eight were incorrectly randomized, two had no followup information, eight had received prior irradiation to areas of measurable disease, five had concurrent second malignancies, one had small cell anaplastic histology, nine had unevaluable disease, and four had

TABLE 1.—Dose schedules and cell types tested for the individual drug regimens

Treatment	Drugs	Dose schedule	Cell types tested
E	CYT + CCNU		SQ, AD, LC
	Cyclophosphamide	700 mg/m² iv q3wks	
	CCNU	70 mg/m² orally q6wks	
J	Dactinomycin	2.5 mg/m² iv q3wks	SQ
K	Dianhydrogalactitol	30 mg/m² iv daily × 5, q3wks	SQ
L	Mitomycin	20 mg/m² iv q3wks	SQ
M	MACC		SQ, AD
	Methotrexate	40 mg/m² iv q3wks	
	Doxorubicin	40 mg/m² iv q3wks	
	Cyclophosphamide	400 mg/m² iv q3wks	
	CCNU	30 mg/m² orally q3wks	
N	CYT + PLAT + BLEO		SQ
	Cyclophosphamide	500 mg/m² iv, Days 1 and 8, q4wks	
	Cisplatin	30 mg/m² iv, Days 1 and 8, q4wks	
	Bleomycin	20 mg/m² iv, Days 1 and 8, q4wks	
O	DBD + ADR		AD, LC
	Mitolactol	140 mg/m² orally daily × 10, q4wks	
	Doxorubicin	45 mg/m² iv q4wks	
P	Ftorafur	4 g/m² iv q2wks	AD, LC
Q	Piperazinedione	15 mg/m² iv q3wks	AD, LC
R	ADR + 5FU + PLAT		AD, LC
	Doxorubicin	25 mg/m² iv, Days 1 and 8, q4wks	
	5-FU	400 mg/m² iv, Days 1 and 8, q4wks	
	Cisplatin	30 mg/m² iv, Days 1 and 8, q4wks	

inadequate renal, hepatic, or marrow function. Selected prognostic factors were distributed by treatment as noted in table 2.

Response

A total of 397 of the 415 eligible patients were evaluable for response. In 12 patients, the lesions were not measured except at the time of initial entry in the study. Consequently, the response status of these patients could not be documented. In six patients, gross underdoses of the drugs were given. All treatment comparisons have been made with respect to treatment E (CYT + CCNU). Table 3 shows the raw objective response rates by treatment and cell type. A more accurate means of making treatment comparisons is to employ a linear logistic model (7) to simultaneously correct for imbalances in patient characteristics due to cell type and the other prognostic factors outlined in table 2. Using this approach, treatment N (CYT + PLAT + BLEO) and treatment R (ADR + 5FU + PLAT) have significantly higher response rates (23%, $P = 0.02$; and 24%, $P = 0.006$; respectively) than treatment E. Treatment L (mitomycin) was marginally active in squamous cell cancer ($P = 0.06$). CYT + CCNU was essentially inactive (9%) as was the MACC regimen (12%). Ambulatory patients responded significantly better than nonambulatory patients ($P = 0.027$). The median time to response was 5 weeks and the median duration of response was 13 weeks. There were no significant differences in response between cell types (SQ, 10%; AD, 11%; LC, 10%) or by extent of disease (limited, 13%; extensive, 10%).

TABLE 2.—% distribution of eligible patients by treatment and selected prognostic factors

Prognostic factor	Level of factor	Treatments* E $n = 81$	J $n = 48$	K $n = 27$	L $n = 28$	M $n = 47$	N $n = 29$	O $n = 41$	P $n = 37$	Q $n = 30$	R $n = 47$
Histologic subtype	SQ	43†	100	100	100	59	100	—	—	—	—
	AD	42	—	—	—	41	—	83	92	90	87
	LC	15	—	—	—	—	—	17	8	10	13
Performance status	Ambulatory (0,1)	68	69	70	71	64	69	71	54	80	81
	Nonambulatory (2,3)	32	31	30	29	36	31	29	46	20	19
Sex	Males	74	81	81	82	77	76	71	57	70	66
	Females	26	19	19	18	23	24	29	43	30	34
Stage‡	Limited	27	42	33	36	28	48	24	22	27	28
	Extensive	73	58	67	64	72	52	76	78	73	72
Wt loss in previous 6 mos (% total)	0–5%	65	56	74	68	66	83	71	73	83	74
	5%–10%	18	25	15	18	19	17	27	14	14	15
	> 10%	17	19	11	14	15	0	2	14	3	11
Prior radiotherapy	Yes	41	29	33	18	30	41	34	27	33	23
	No	59	71	67	82	70	59	66	73	67	77
Prior surgery	Yes	37	17	30	46	32	41	27	24	37	40
	No	63	83	70	54	68	59	73	76	63	60
Time from 1st symptoms to start of therapy	≤ 6 mos	68	75	70	65	56	50	81	79	66	53
	> 6 mos	32	25	30	35	44	50	19	21	34	47
Age	< 50 yrs	18	11	19	5	12	7	17	21	10	15
	50–60 yrs	39	43	41	38	22	21	44	35	38	38
	60–70 yrs	38	39	30	38	44	65	29	35	52	36
	> 70 yrs	5	7	10	19	22	7	10	9	0	11

*Actual drug regimens are listed in table 1.

†For each treatment, values = % of patients in each prognostic subgroup.

‡Limited refers to disease confined to the ipsilateral hemithorax including supraclavicular nodes. Extensive refers to spread beyond these margins.

Survival

All 415 eligible patients are included in this analysis. Survival time is measured from date of randomization to date of death or to the date the patient was last known to be alive in the case of censored observations. The estimated median survival by treatment and cell type is given in table 4. The median survival for patients receiving treatment R (ADR + 5FU + PLAT) appears superior (27.4 and 26.3 weeks for patients with AD and LC carcinoma, respectively). This interpretation is, however, subject to a number of potential biases because the different treatments being compared have different mixes of the prognostic factors which affect survival. For example, 38 of 47 patients (81%) receiving treatment R were ambulatory compared to 55 of 81 patients (68%) receiving treatment E. Using a Cox modeling technique (8) similar to the linear logistic model used to evaluate response, we can make treatment comparisons while adjusting for the effects of the known prognostic factors. When all other effects are taken into account, none of the treatments is significantly better for survival than treatment E (CYT + CCNU). Ambulatory status ($P < 0.0001$), limited disease ($P = 0.0004$), and prior surgery ($P = 0.002$) are significant positive prognostic variables whereas prior weight loss ($P = 0.0003$) and prior radiotherapy ($P = 0.02$) have a deleterious effect on survival. Responders have a significantly improved median survival of 31.6 weeks versus 15.7 weeks for nonresponders ($P = 0.002$).

Toxicity

Complications due to therapy are routinely monitored on a six-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, and 5 = drug-related death). Of the 415 eligible patients, 399 were evaluable for toxicity. The six untreated patients and ten patients with insufficient records to document toxicity were excluded. Table 5 lists the combined number of severe, life-threatening, and fatal toxic reactions for each type of toxicity and each treatment. Treatments L (mitomycin), M (MACC), and Q (piperazinedione) had signifi-

TABLE 3.—Objective tumor response by treatment and cell type

Cell type	Treatment*	No. of patients treated	No. of patients by response category			Total response rate (CR + PR), %
			CR	PR	No change or disease progression	
SQ	E	34	0	2	32	6
	J	47	0	3	40	6
	K	26	0	0	22	0
	L	27	0	5	28	19
	M	28	0	4	23	14
	N	26	0	6	20	23
	Total	188	0	19	169	10
AD	E	32	0	3	29	9
	M	15	0	1	14	7
	O	32	1	2	29	9
	P	33	0	1	32	3
	Q	27	0	2	25	7
	R	39	0	10	29	26
	Total	178	1	19	158	11
LC	E	12	0	2	10	17
	O	7	0	0	7	0
	P	3	0	0	3	0
	Q	3	0	0	3	0
	R	6	0	1	5	17
	Total	31	0	3	28	10

*Exact treatment regimens are listed in table 1.

TABLE 4.—Estimated median survival by treatment and cell type

Cell type	Treatment*	Estimated median survival (wks)
SQ	E	12.5
	J	16.1
	K	11.9
	L	12.1
	M	13.7
	N	18.1
	Total	15.3
AD	E	15.1
	M	13.9
	O	24.0
	P	13.7
	Q	15.3
	R	27.4
	Total	17.7
LC	E	20.3
	O	15.8
	P	5.2
	Q	10.4
	R	26.3
	Total	18.4

*Exact treatment regimens are listed in table 1.

cantly more life-threatening and lethal toxic effects than the other treatments (joint P value < 0.0001 using Fisher's exact test) leading to the early termination of the latter two regimens. Overall, the bulk of life-threatening problems were related to hematologic toxicity. Of ten drug-related deaths, seven were due to bone marrow toxicity, one to bleomycin-induced pulmonary fibrosis, one to hepatic failure, and one to pulmonary hemorrhage and hypotension of unclear etiology. Responders did not have more toxic reactions than nonresponders.

DISCUSSION

Successful treatment of advanced non-small cell bronchogenic carcinoma remains an elusive goal. A critical problem has been the difficulty in evaluating new agents and regimens. Animal studies infrequently correlate with clinical results and there are large discrepancies between single institution and cooperative group results. Many studies, including the present one, rely heavily on preliminary reports in small groups of patients. Review of the individual regimens in this study may lend impetus to at-

TABLE 5.—Analysis of toxicity by treatment*

Type of toxicity	No. of patients with severe, life-threatening, or fatal toxic reactions by treatment†									
	E $n = 78$	J $n = 48$	K $n = 26$	L $n = 27$	M $n = 43$	N $n = 26$	O $n = 39$	P $n = 36$	Q $n = 30$	R $n = 46$
Gastrointestinal‡	1	2	1	0	4	0	1	0	0	1
Vomiting	7	11	0	0	9	3	2	6	2	14
Diarrhea	0	0	0	0	0	0	1	0	0	0
Infection	0	0	1	0	3	0	0	0	0	0
Bleeding	1	1	1	1	4	0	1	0	2	2
Skin or mucous membrane	0	1	0	0	0	0	1	0	0	0
Neurologic	1	0	1	0	2	1	2	3	1	2
Respiratory	0	0	1	1	0	1	1	0	0	1 (1)
Genitourinary	0	0	0	0	0	0	0	0	0	2
Hepatic	0	1	0	0	1	0	2	0	0	0
Hematopoietic	10	5	4 (1)	9 (1)	21 (2)	5	12 (1)	0	14 (1)	11 (1)
Drug deaths	0	0	1	1	2	1	2	0	1	2

*Treatment regimens are listed in table 1.
†Nos. in parentheses = No. of deaths.
‡Other than vomiting.

tempts to avoid groupwide testing of regimens before completion of pilot studies in adequate numbers of patients.

CYT + CCNU was chosen as standard therapy on the basis of both prior experience in small groups of patients and suggestive animal data. Bodey et al (9) reported four responses to CYT + CCNU among ten patients with lung cancer, and the combination was clearly superior to either agent alone in small cell anaplastic carcinoma, although there was no benefit in patients with AD (10). Synergistic activity in the Lewis lung model suggested a possible therapeutic advantage for the combination (11). Our overall response rate of 9% for this combination (total cases, 140) demonstrates a lack of clinical utility. Other studies have confirmed the lack of activity for this regimen in non-small cell lung cancer (12,13).

Dianhydrogalactitol and the MACC regimen were tested on the basis of enthusiastic reports from single institutions. Eagan et al (14) preliminarily reported four responses to dianhydrogalactitol among seven patients with epidermoid lung cancer, but the actual response rate dropped to 15% for epidermoid cancer and 11% overall when the final results were published later (15). We saw no responses with dianhydrogalactitol in 26 patients treated with the same regimen. Chahinian et al (16) preliminarily reported a 42% response rate to MACC (four responses among nine patients with squamous cell cancer). A later abstract in 1977 (17) continued to suggest significant activity (47% response rate, including 12 CRs or PRs among 30 patients with non-small cell carcinoma) with acceptable toxicity as did the final manuscript (18). Our response rate of 12% and the severe toxicity encountered make it unlikely that this is a useful regimen for the treatment of non-small cell lung cancer.

Ftorafur (19), dactinomycin (20), and mitomycin (21) were employed after animal and human testing suggested that a change in dose scheduling would either reduce toxicity or improve response rates. Piperazinedione was a new agent being used in initial phase II testing in non-small cell lung cancer.[5] Mitomycin demonstrated clinical activity (19%) in squamous cell cancer, but the other three agents had virtually no activity and are unlikely to be useful in further combinations. Mitomycin-containing regimens are being evaluated in generations IV and V of EST 2575.

The three other combination regimens were selected primarily on the basis of promising single-agent data. CYT + PLAT + BLEO was chosen because of the single-agent response rates of cyclophosphamide (22,23) and bleomycin (24). Although cisplatin had minimal activity in lung cancer (25), its addition was based on the synergism exhibited with cyclophosphamide in animal systems (26). Doxorubicin was an obvious choice for the ADR + 5FU + PLAT and DBD + ADR regimens as one of the more active single agents in lung cancer (22,27,28). Trials of ADR + 5FU (29,30), ADR + PLAT (15,31), and ADR + DBD (32) also suggested activity in a number of cancers. It is of interest that the two cisplatin-containing arms demonstrated the most responses: 23% for CYT + PLAT + BLEO and 24% for ADR + 5FU + PLAT. As late as 1977 cisplatin was considered an inactive single agent in lung cancer (33), although it demonstrated activity as part of the cyclophosphamide-doxorubicin-cisplatin (CAP) regimen of Eagan et al (15). The chemotherapeutic synergy seen in animal systems (26) may be related

[5]Straus M, and Slavik M. Clinical brochure: piperazinedione. Prepared by the National Cancer Institute, Bethesda, MD, 1975.

to the numerous reports of hypoxic cell sensitization and radiotherapeutic enhancement in a number of animal and in vitro systems (34). While the cisplatin-containing regimens themselves demonstrated only marginal clinical benefit (improved response rates, but no increase in survival), they point the way to further studies to optimize the timing and dose of cisplatin with respect to other agents. The recent reports of significant activity in lung cancer when vindesine is combined with cisplatin (35) also highlight possible synergistic interactions.

One element of confusion in interpreting preliminary reports concerns the use of response rates. Institutions that employ a "minimal response" or "objective regression" category will, by definition, have a higher response or regression rate. Eagan et al (6) reported that there may be no difference in survival when these two systems are compared, and the less rigorous definition of "regression" is often better suited to the indistinct lesions associated with lung cancer. However, when comparisons are made without indicating which response categorization is being used, confusion reigns. Although we in ECOG prefer the more conservative CR and PR criteria, there is a critical need for universally accepted definitions. Our analysis also stresses the need for reasonably large groups of patients when some responses are seen. Performance status, extent of disease, weight loss, prior radiotherapy, and prior surgical resection all significantly influence response or survival, and it is important to use sophisticated statistical models to account for these various prognostic factors.

One could argue that patients with limited disease should have received radiotherapy rather than chemotherapy, which showed no difference in response rates over patients with extensive disease (13% vs 10%) and a significant but modest effect on survival (22 vs 14.8 weeks). Although better response rates and survival in this group of patients have been reported for radiotherapy trials [*see* review by Bleehen (36)], the results cannot be directly extrapolated. Many of the patients with limited disease randomized to EST 2575 were otherwise medically unable to undergo radiation therapy and some had demonstrated intrathoracic disease progression following prior radiotherapy.

The 31.6-week median survival of responders is not drastically different than that of patients with extensive small cell anaplastic cancer, and with the ≥ 25% of patients responding to HAM (5), CAMP (37), vindesine plus cisplatin (35), CAP (15), and the two cisplatin-containing regimens from this study, we appear to be making some inroads in the medical therapy of inoperable non-small cell lung cancer. A 31.6-week median survival in one fourth of the patients is, however, not sufficient. We plan to complete testing the regimens with reported activity in all three histologic subtypes to develop a standard therapy for non-small cell lung cancer against which further testing can be done.

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Immediate Versus Postponed Combination Chemotherapy (CAMP) for Unresectable Non-Small Cell Lung Cancer: A Randomized Trial [1]

Thomas E. Lad,* Rodney B. Nelson, Ulrich Diekamp, Lawrence J. Kukla, P. R. Sarma, Carl S. Larson, Evelyn T. Currie, Manjeet S. Chawla, Thomas Tichler, Patricia Zawila, and William P. McGuire, III [2]

SUMMARY

A randomized control trial was performed in good performance status patients with unresectable non-small cell lung cancer to test a strategy of early aggressive combination chemotherapy (CAMP [cyclophosphamide, doxorubicin, methotrexate, and procarbazine]) versus a strategy of delaying such treatment until clinical deterioration. Thirty-seven patients received immediate CAMP and 35 patients received initial low-dose single-agent CCNU (CAMP was postponed). Immediate CAMP therapy produced an objective response rate of 44% in patients with measurable lesions, and CCNU produced none. Median survival was 193 days for the immediate-CAMP group and 175 days for the postponed-CAMP group ($P = 0.26$). Measures of quality of life were made and no difference emerged between the two treatment strategies. This trial failed to show substantial benefit from immediate combination chemotherapy in minimally symptomatic patients with non-small cell lung cancer.

[Cancer Treat Rep 65:973–978, 1981]

Combination chemotherapy regimens containing doxorubicin can cause tumor regression in 30%–40% of patients with non-small cell lung cancer (1–7). The CAMP combination is one of these regimens and consists of cyclophosphamide, doxorubicin, methotrexate, and procarbazine (2–4). The benefit of these regimens has been assessed through the use of historic controls. When we compared survival times of our CAMP-treated patients with historic controls matched by age, stage, and performance status, we found no difference. A more rigorous method of treatment assessment is the randomized prospective control trial.

Our study design was a compromise among physicians with differing views of the lung cancer chemotherapy data available at the time. We agreed that radiation therapy had not provided significant clinical benefit for patients with lung cancer when tested against no treatment in the Veterans Administration randomized trial, since the survival difference was significant at 0.05 only by using a one-tailed test (8). A randomized trial of combination chemotherapy versus no treatment was not acceptable to all members of our group because of ethical considerations based, in part, on fear of an adverse psychologic effect in patients randomized to receive no treatment. Our study was designed to offer hope to all patients even though one half were to receive initial treatment (ie, low-dose CCNU as a single agent) considered by some to be little more than a placebo (1). Placebo effect, coupled with the promise of further possibly effective treatment, when necessary, may have great benefit for a patient with a serious illness. However, at the time of study design, we were not in agreement as to whether low-dose CCNU could be assumed to be either nontoxic or completely inactive.

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[2] Medical Oncology Section, Department of Medicine, West Side Veterans Administration and University of Illinois Hospitals, Abraham Lincoln School of Medicine, Chicago.

Reprint requests to: Thomas E. Lad, MD, Department of Medicine, University of Illinois Hospital, PO Box 6998, Chicago, IL 60680.

METHODS

The study population consisted of patients who fulfilled the following criteria: stage III M_0 or M_1 non-small cell lung cancer, a Zubrod performance score of 2 or better, and informed consent. To assure reproducibility of performance status assignment, performance status 3 was defined as housebound, not including visits to the clinic.

Over a 26-month period, 72 patients were randomized to receive initial treatment with either CAMP combination chemotherapy (4) or 50 mg/m² of CCNU as a single agent every 4 weeks. The CAMP-treated patients continued to receive CAMP until deterioration ("E_1," defined below) or death. Treatment after CAMP failure was at the discretion of the primary physician. CCNU-treated patients continued to receive CCNU until E_1, when they were crossed over to CAMP. E_1 was defined as one of four events: deterioration to housebound status (Zubrod performance score of 3), loss of 12% body weight, progression of tumor by volume doubling or appearance of new metastases, or death.

Patients were substaged at the time of randomization as having "limited" or "extensive" disease, as defined by the Veterans Administration Lung Cancer Study Group (9). Radiation treatment of the primary tumor was given only for palliation of symptoms. Primary radiation therapy was completed prior to randomization if given before chemotherapy. Twelve of the 34 patients with limited disease were treated in this manner. Response to chemotherapy was assessed according to Eastern Cooperative Oncology Group criteria (4). "Stable" patients were not considered responders.

Survival from the day of randomization was determined for each patient. Also determined were time to E_1 and time to housebound status (for patients whose E_1 was weight loss or tumor progression). These times were thought to reflect quality of life; thus, the influence of combination chemotherapy on the quality of life could be determined independent of survival. Balanced, nonstratified randomization was used. Our experience indicated that we would be able to enroll approximately 30 patients/year, and that median survival would likely be about 6 months. We then estimated that about 2.5 years would be necessary to enroll about 70 patients and that after 40 deaths the trial would have a power (type II risk) of about 0.5 for detecting a difference ("delta") of 0.37. In other words, we accepted a limited ability to detect a small benefit because of the toxicity and expense we felt inherent in CAMP treatment. The data were analyzed three times: once after 1 year (4), once when patient accrual ended (10), and here, when all but five patients were dead. A total of four tests of significance comparing CAMP with CCNU were performed utilizing the log-rank test (11). All significance levels are two-sided.

RESULTS

Of the 72 patients, 37 received CAMP and 35 received CCNU as initial treatment. Of the CCNU patients, 23 subsequently received CAMP, and 12 patients (34% of the CCNU group) died without ever receiving CAMP. At the time of analysis only five patients were alive, and two of these had not reached a deterioration point (E_1). No patient was lost to followup. Table 1 shows the characteristics of the two groups. A trend toward unbalanced randomization of some prognostic variables is evident. None of these reached significance and tend to cancel each other in that some of the unbalancing favored one group and some the other.

Thirteen of the 43 patients with measurable disease receiving CAMP achieved an objective response, one of which was complete. The survival advantage of responders approached significance (median, 347 vs 153 days; $P = 0.11$). Of these 43 patients, 25 received immediate CAMP (11 responders, 44%) and 18 received CAMP after reaching E_1 on CCNU treatment (two responders, 11%). Another five patients, who did not have measurable lesions, received CAMP after E_1. Three of these patients improved their performance status on CAMP. No response to CCNU was seen. Thus, 11 of the 37 patients randomized to receive immediate CAMP achieved objective response (30%) and two of the 35 patients randomized to receive initial treatment with CCNU were responders (6%).

Despite the absence of objective response to CCNU, no differences existed in time to death, time to deterioration, or time to housebound status between the immediate-CAMP and initial-CCNU groups (figs 1–3).

Also, among patients with limited-stage disease, the survival difference was not significant between the two treatment groups (median, 385 vs 253 days; $P = 0.7$). Anatomic substage did not quite reach significance as a prognostic factor. Histologic type did not influence survival. CAMP was well-tolerated (table 2) and no drug-related toxicity was encountered from CCNU.

The size of this trial (67 deaths) gives an 80% chance of detecting a survival difference of about 40% between the two groups (ie, the type II risk is about 0.20 with a two-tailed type I risk of 0.05). A 40% difference roughly means that 70% of patients in the better treatment group would be alive when

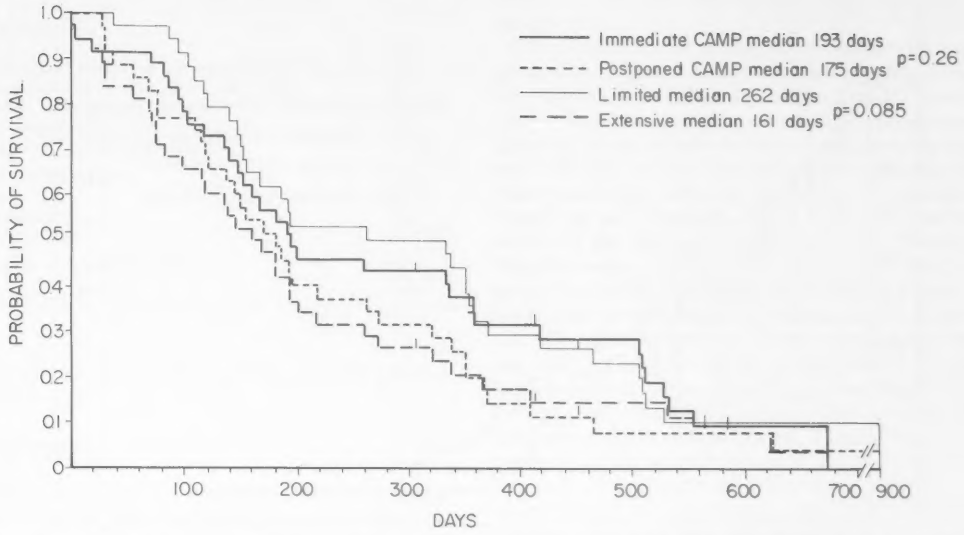

FIGURE 1.—Survival from randomization according to treatment assignment and stage. Small vertical marks = patients who remain alive at the time of analysis.

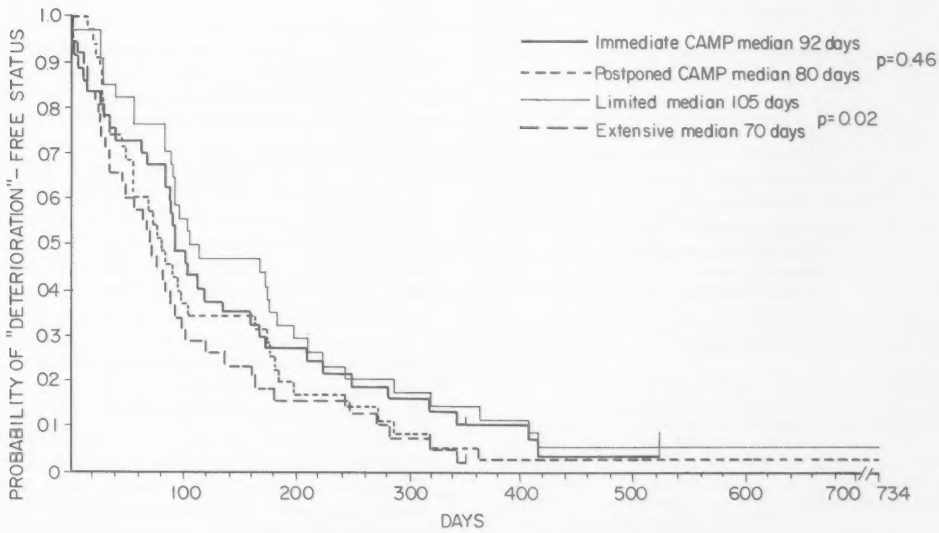

FIGURE 2.—Time from randomization to deterioration (E_1) according to treatment assignment and stage. Small vertical marks = patients who have not yet reached E_1.

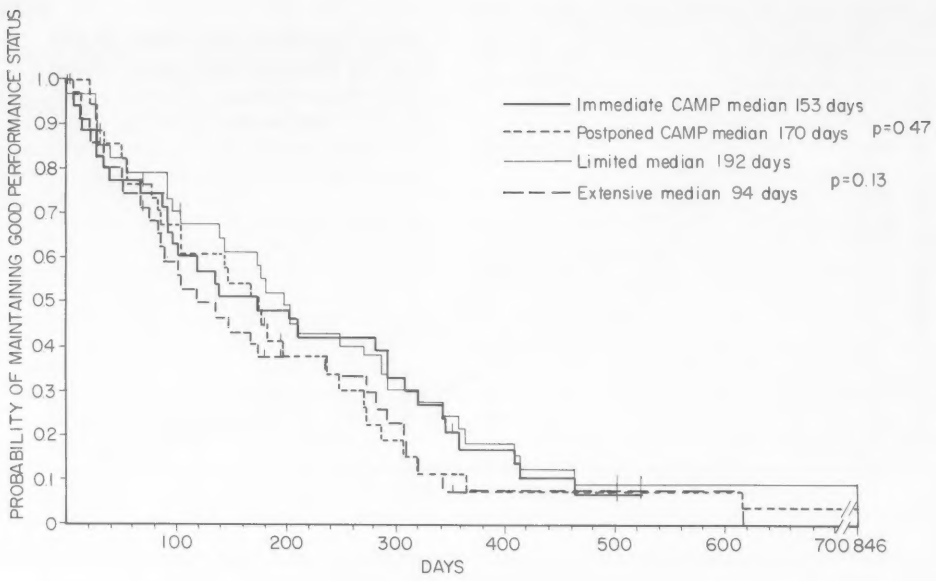

FIGURE 3.—Time from randomization to housebound status according to treatment assignment and stage. Small vertical marks = patients who have not yet reached poor performance status, including those who died without becoming housebound.

TABLE 1.—Randomization results*

Prognostic factor	Initial treatment		Total (100%)
	CAMP (%)	CCNU (%)	
Stage			
Limited	20 (59)	14 (41)	34
Extensive	17 (45)	21 (55)	38
P value†	0.34		
Cell type			
Squamous cell carcinoma	23 (55)	19 (45)	42
Adenocarcinoma	7 (47)	8 (53)	15
Large cell undifferentiated carcinoma	7 (47)	8 (53)	15
P value†	0.79		
Performance status			
0	5 (30)	12 (70)	17
1	22 (61)	14 (39)	36
2	10 (53)	9 (47)	19
P value†	0.10		
Prior radiotherapy (for symptomatic disease)			
Yes	8 (38)	13 (62)	21
No	29 (57)	22 (43)	51
P value†	0.23		
Total	37 (51)	35 (49)	72

* Unless otherwise specified, values = No. of patients.
† Chi-square test.

TABLE 2.—CAMP tolerance

No. of patients—	
Total	72
Randomized to receive immediate CAMP	37
Randomized to receive postponed CAMP	35
Died without receiving CAMP	12 (34%)
Receiving CAMP	60
Unable to tolerate procarbazine	3 (5%)
No. of CAMP cycles—	
Total	265
Mean/patient	4.4
At 100% dose	167 (63%)
Total dose of CAMP received	85%
Mean wks/CAMP cycle	4.2

the median survival of the poorer survival group is reached (11).

DISCUSSION

This study can be considered an "aggressive chemotherapy versus minimal treatment trial" for two reasons. First, nearly one third of the patients randomized to receive initial treatment with low-dose CCNU never received combination chemotherapy. Second, after cross-over of CCNU patients to CAMP the response rate was low, probably because of poor performance status. Poor performance status is known to predict poor response to treatment.

What appears to be a discrepancy between the survival advantage for CAMP responders and the lack of overall superiority of immediate CAMP deserves comment. The validity of retrospective comparisons by response and compliance can be questioned on logical and empiric grounds (12), and much of the evidence favoring combination chemotherapy is based on responder/nonresponder analysis. In addition, this trial was designed to quantitate the effect of combination chemotherapy on quality of life and survival rather than on tumor regression. Indeed, tumor measurability was not among the criteria for inclusion. The endpoints used in this trial (ie, death and other clinical deterioration) were easily determined, and thus suitable to the methodology of prospective, randomized trials.

Our survival curves are observations rather than predictions (ie, not heavily censored since all but five patients have died). In fact, our figures calculated at cessation of accrual to this study showed a significant survival advantage for limited-stage patients and CAMP responders (10). These significant differences did not persist. The fact that different conclusions may thus be reached within the same study depending on the length of followup illustrates the problems arising from early reporting of results (11).

The sensitivity, or power, of this small trial is not great (13), but three conclusions can be made. First, the magnitude of clinical benefit of CAMP combination chemotherapy for non-small cell lung cancer is not as large as historic control comparisons suggest (2). The median survival of our patients was about 6 months, whether they received immediate CAMP or not. Although this is almost double that observed for untreated patients in the Veterans Administration studies (9), our patients were selected for initially favorable performance status, as is the case in most cancer treatment trials. Second, CAMP combination chemotherapy at full doses does not have an adverse effect on survival of patients with non-small cell lung cancer, as was found in the Oxford trial with MVPP (14). In fact, combination chemotherapy is well-tolerated. Finally, care must be taken in the interpretation of chemotherapy experience citing survival differences between responders and nonresponders. Our CAMP results in terms of response rate and survival of responders are comparable to results from other studies using the same regimen. However, the survival and quality of life of our postponed-CAMP group (6% responders) and our immediate CAMP group (30% responders) were not different, although response was an indicator of a favorable prognosis. Future studies of combination chemotherapy should incorporate quality of life assessment in addition to the standard measurements of response rate and survival.

The survival and quality of life of patients with non-small cell lung cancer who have good performance status are quantitated in this study, and little benefit from immediate combination chemotherapy is evident. Such treatment for patients with unresectable non-small cell lung cancer with minimal symptoms should not be considered beneficial until well-controlled trials demonstrate improvement in the quality of life or a survival advantage.

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Commentary: Current Status of Chemotherapy for Non-Small Cell Lung Cancer[1]

Joseph Aisner [2,*] and Heine H. Hansen [3]

Combination chemotherapy has proven useful for a variety of human malignancies, producing dramatic antitumor responses, prolongation of median survival, and in some diseases long-term disease-free survivors. Furthermore, the identification of active combinations in advanced disease has led to use as adjuvants to surgical procedures with resultant improvement in disease-free and overall survival. One of the successful applications of combination chemotherapy has been in small cell lung cancer where combination chemotherapy has produced an improvement in median survival and a small fraction of long-term disease-free survivors (cures?). It is therefore not surprising that some of the enthusiasm associated with the treatment of small cell lung cancer should find its way into the treatment of non-small cell lung cancer (epidermoid carcinoma, adenocarcinoma, large cell undifferentiated carcinoma, and mixed histologies).

In recent years, many combination chemotherapy regimens have been studied for the treatment of non-small cell lung cancer and a partial list is shown below by abbreviations (acronyms are not necessarily those of the reporting authors).

ABCM	CA	DAP	MACC
ACMV	CAMP	EP	MVPP
BACON	CAP-I	FACP	NAC
COMB-I	CAP-II	FAM	PACCV
COMB-II	CAP-B	FCC	V-CAP
BMS	CCAPHM	HAM	VAB
C-MOPP	CCM	HBC	VP

Therapeutic results of these combinations have conflicted even when various investigators or groups used the same combination. Initial reports for some of these combinations showed highly encouraging results, with partial response rates varying from 30% to > 40% (1–8). However, followup studies by other investigators, or randomized trials testing these combinations against a single agent, showed a much lesser activity with only minor response rates, usually < 10% (9–12). Furthermore, toxicity from these combinations was frequently found to be considerable or excessive in view of the minimal response. In addition, there has been a certain lack of reproducibility of results for some combinations (5,10). For example, in one set of cooperative group studies a given combination (HAM) was found to have a response rate of 27% (13). In a subsequent study by the same group (14), HAM was compared to the CAMP combination but the response rate to the HAM regimen was 13%, or less than one half of that in the original study. The response frequency of the CAMP regimen (22%) was found to be higher than that of the HAM regimen but was in reality similar to the response of the HAM combination (27%) in the first study.

Certainly the majority of these studies were carefully conducted and carefully analyzed, yet the total of these studies produces conflicting results. These often contradictory data have led to disagreement and uncertainty regarding the role of combination chemotherapy in non-small cell lung cancer. This would suggest that other factors that are not uniformly considered in the evaluation of the treatments may be involved in the determination of response and survival. The purpose of this paper, therefore, is to review the methods of analysis and the factors that influence response and survival.

RESPONDERS VERSUS NONRESPONDERS

One of the most common methods of analyzing the results of treatment for non-small cell lung cancer is to compare the survival for responders and nonresponders. A set of survival curves for the theoretic chemotherapy combination "XYZ" is illustrated in figure 1. Using such a comparison, an author will frequently find a statistically significant difference between the two groups depending on such variables as size of the population and the duration of followup. With a "significant P value," the author then comes to the logical conclusion that the treatment was responsible for the difference and is, therefore, worthwhile. A corollary of this conclusion

[1]Received Aug 13, 1981; accepted Aug 17, 1981.

[2]Medical Oncology Section, Baltimore Cancer Research Center, University of Maryland Hospital.

[3]Chemotherapy Department RII-V, Finsen Institute, Copenhagen, Denmark.

**Reprint requests to:* Joseph Aisner, MD, Baltimore Cancer Research Center, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

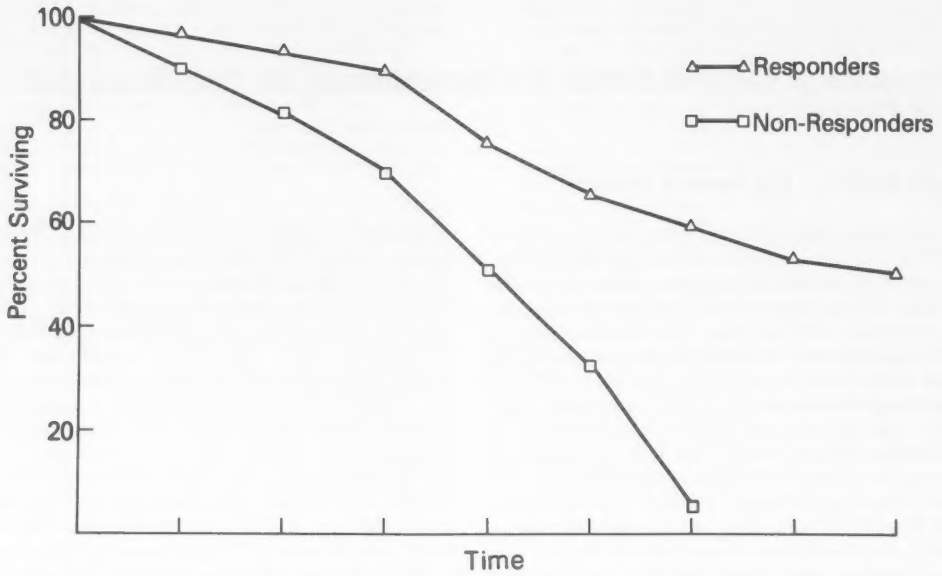

FIGURE 1.—Hypothetic survival curves modeled after many reports showing the effect of response to theoretic combination XYZ on survival in non-small cell carcinoma. This curve shows that the responders do better than the nonresponders, a feature common to most human malignancies. The figure, however, does not take into account the various prognostic factors that determine survival.

is that a fraction of the population, ie, patients who respond to XYZ, benefits from that treatment. There are, however, several difficulties with this interpretation. It is conceivable that there are other factors, both recognized and yet to be identified, that determine response or survival or both and that are independent of the drug treatment. These factors are generally not completely taken into consideration in the simple comparison of responders and nonresponders. Comparison of responders and nonresponders may simply be a means of selecting a group of patients who are more likely to respond and survive longer or vice versa. Is there any information available which suggests that there are such factors and that we could identify at least some of these factors prospectively?

PROGNOSTIC FACTORS FOR RESPONSE AND SURVIVAL

Nearly all human malignancies have prognostic factors that determine or predict the clinical behavior of the disease. In lung cancer a number of prognostic factors are well-recognized (15–17). For small cell lung cancer, these include performance status and extent of disease and may also include sites of involvement and response to treatment. For non-small cell lung cancer, multiple prognostic factors include initial performance status, extent of disease, presence of weight loss, and possibly prior therapy as well as others such as histologic subclassification (15–17). Although many of these factors may be interrelated, comparison of therapies for non-small cell lung cancer must take into account the distribution of these prognostic factors. Failure to recognize or account for the prognostic variables increases the likelihood of producing either false-positive or false-negative interpretations. Thus, for any comparisons (eg, responders versus nonresponders or treatment A versus treatment B), it is important that each side of the comparison have a similar "mix" of each of the prognostic factors. In fact, when one examines many of the comparisons of responders and nonresponders, one generally finds that the responders constitute a group with more favorable prognostic factors whereas the nonresponders have the less favorable factors.

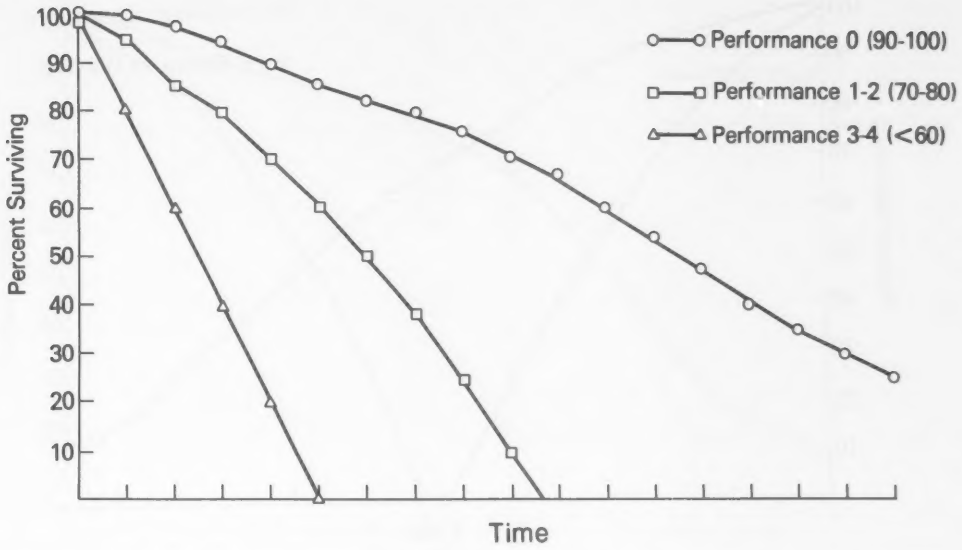

FIGURE 2.—A hypothetic survival curve modeled after VALG and similar data illustrating the effect of performance status on survival in non-small cell lung cancer. This graphically illustrates that the better the performance the greater the survival.

In order to evaluate the relative importance of the prognostic factors in determining response and survival, a large population base must be available so that appropriate statistical maneuvers such as the log-rank analysis or multivariate (step-down) analyses can be performed. Most of the relatively recent reports on the use of various combination chemotherapies contain too few patients for such analyses to be undertaken. The best information for this purpose is in the Veterans Administration Lung Group (VALG) studies Nos. 9–15, which entered > 5000 patients with inoperable bronchogenic carcinoma of the lung (15,18). Some of these studies are > 10 years old and the chemotherapy data may be somewhat limited. However, the data that can be derived from the prognostic factors identified in these studies remain valid and must be addressed in future prospective trials.

Performance Status

The performance status is a discrete measure of a patient's ability to ambulate and care for self. There are many scales for performance status including the ten-point scale (0–100%) of Karnofsky et al (19), the five-point scale of Zubrod et al (20), and the four-point scale of the Cancer and Leukemia Group B (21). To simplify the sometimes subjective nature of the ten-point scale, many investigators have either chosen smaller scales or have condensed the ten-point scale into groups (8). For example, some studies have grouped all patients with a performance status ≥ 70, while others utilized an even broader grouping. Figure 2 illustrates the types of effect that performance status can have on survival in non-small cell lung cancer. These theoretic curves show graphically the relatively distinct differences in survival that are seen according to progressive impairment of the patient's ability to ambulate and care for self. In nearly every investigation in which performance status has been considered, it has been found to be one of the most, if not the most, important prognostic factor in determining survival for patients with non-small cell lung cancer (15–17). In fact, when performance status is graphed on the ten-point Karnofsky scale, differences exist in survival between a performance status of 70% and one of 80%. This was recently demonstrated by Stanley (15), who in analyzing the large VALG accrual (> 5000 patients) used a recoded scale and showed a statistically significant linear relationship between the log of the median survival and the rescaled performance status. Such a finding suggests that stud-

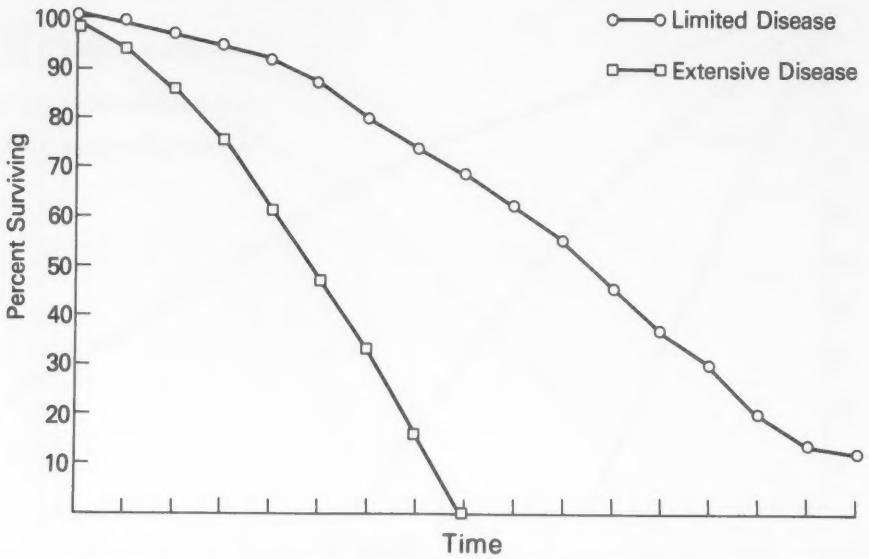

FIGURE 3.—Hypothetic survival curves modeled after VALG and similar data that show the effect of extent of disease on survival in non-small cell lung cancer. Patients with extensive disease survive for a shorter period than those with limited disease. Within the category of extensive disease there may be subcategories of sites of disease which may further define prognostic features of this category.

ies which collapse the performance status into larger groups may allow for certain degrees of imbalance in this highly significant prognostic factor, and this may override the effects of the treatments.

Extent of Disease

In addition to the usual TNM classification and the I, II, III, (IV) staging system, lung cancer has also been classified according to limited and extensive disease. This latter classification system lends itself well to radiation therapy port planning since limited disease includes one hemithorax with or without mediastinal nodes and with or without ipsilateral supraclavicular nodes. Extensive disease includes all disease beyond the confines of the definition for limited disease. Investigators from the VALG long ago recognized that patients with extensive disease have a significantly worse survival, independent of any treatment, than patients with limited disease (22). Figure 3 is a hypothetic, modeled set of survival curves which shows the type of survival differences that may be seen according to the extent of disease. It is thus immediately evident that any evaluation of survival must also balance the compared groups for extent of disease. Thus, appropriate staging procedures as practiced for small cell lung cancer may be necessary to define prognosis and to properly stratify patients. It is also likely that, in addition to the extent of disease, certain sites of involvement (eg, brain metastases or liver metastases) may define subgroups of extensive disease for which survival is even poorer. Such a difference according to sites of metastases has recently been suggested for small cell lung cancer (23), and it is conceivable that a similar subclassification for extent of disease might be shown for non-small cell lung cancer.

Weight Loss

Studies by the VALG have also clearly indicated that weight loss in the preceding 6 months is an important prognostic factor (15). Figure 4 is a theoretic set of curves which illustrates the type of effect that a $> 10\%$ weight loss may have on survival. In fact, the VALG data show that this prognostic factor can be divided into three categories of body weight loss: none, $< 10\%$, and $> 10\%$ (15). Patients analyzed according to the categories of weight loss showed differing survival. Again, as for the other prognostic factors, an imbalance of this factor in the groups being compared may produce either false-positive or false-negative results.

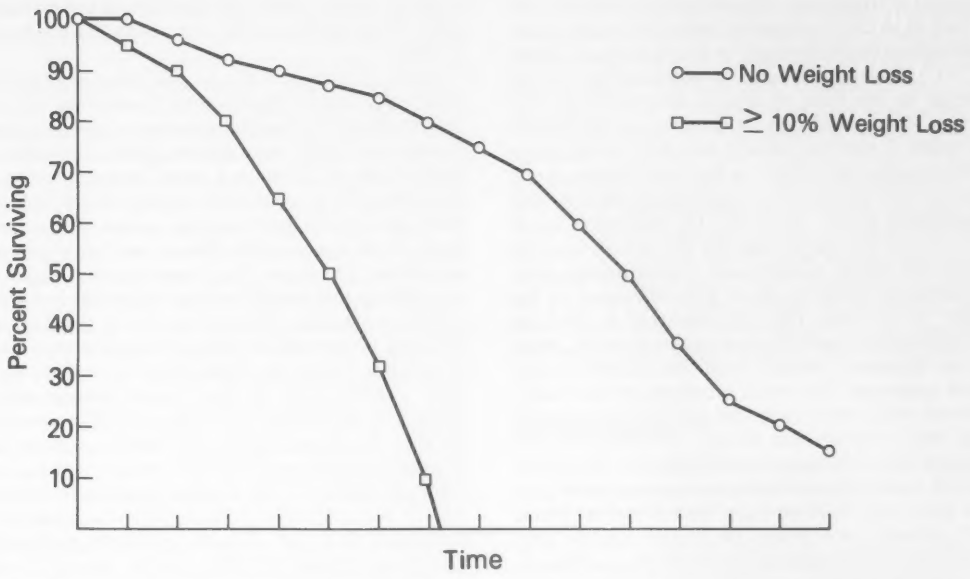

FIGURE 4.—Hypothetic survival curve modeled after VALG and similar data illustrating the effect of weight loss on survival in non-small cell lung cancer. Here patients with > 10% body weight loss in the preceding 6 mos have an inferior survival to those who had no weight loss. An intermediate group exists with a 5%–10% weight loss.

Combinations of Prognostic Factors

Regression analyses have clearly shown that the three factors, in order of importance, are performance status, extent of disease, and weight loss and that they are independent (15). Using a variety of models based on the large VALG accrual, Stanley (15) has estimated that the median survival can vary from 6 to 72 weeks when the three prognostic factors are considered together. For example, for a group of patients with an initial performance status of 100, no evidence of weight loss, and limited disease, a median survival of 72 weeks might be anticipated. For patients with an initial performance status of 70, extensive disease, and no weight loss, a median survival of 26 weeks might be seen, whereas for patients with a performance status of < 50, >10% body weight loss, and extensive disease, a median survival of only 6 weeks might be seen. A table illustrating the median survival estimates in weeks according to these three prognostic factors can be found in the recent publication by Stanley (15). The combination of these factors compounds the difficulty of assuring an equal mix in comparisons of any groups or treatments in all but the largest studies. This makes it nearly impossible to determine if, in fact, a given treatment will have an effect on survival, and comparison of responders and nonresponders should therefore be discouraged as misleading.

Although it is certainly conceivable that one or more of the current combination chemotherapies may have a beneficial effect for the responders, the difficulties with assessing the comparability of the groups precludes a definitive conclusion. Past studies by the VALG failed to show that chemotherapy (albeit much less intense than currently given) had no effect on survival independent of the prognostic factors, and to date no study has convincingly demonstrated such an effect. Furthermore, since total response rates for any given drug or combination are well below 50%, it is unlikely that median survival will be affected. Comparisons of survival at 1 and 2 years may be needed, and these data should be provided. Thus, it still remains to be demonstrated that a given combination with a positive effect (usually defined in response rate) has an effect on any aspect of survival independent of the recognized prognostic factors.

One approach to this problem would be to test a given combination with a "reasonable" response rate against palliative therapy. Considering the necessary constraints of large patient accrual, this would be ideal for a cooperative group study. Should such a study show a beneficial effect on survival despite the prognostic factors, it could be adopted as a

standard of treatment. Interestingly, a recent study by Lad et al (24) randomized patients to immediate combination chemotherapy or low-dose single agent (CCNU) therapy followed by combination chemotherapy at the time of clinical deterioration. The authors followed quality of survival as well as the end point of survival (death) and showed an objective response rate of 44% in the combination chemotherapy and no objective responses in the low-dose single-agent group. However, the authors failed to show a survival advantage for the group who received the initial combination chemotherapy and, furthermore, failed to show any difference in the quality of survival. This suggests that at least for the combination chemotherapy regimen tested, there was no apparent benefit from the objective antitumor response. The small numbers in this study, however, still leave open the question of a totally equal "mix" of prognostic factors. Nevertheless, this supports the investigative strategy for the evaluation of combination chemotherapy regimens that have been identified to have high response rates. Such combinations would be tested against only symptomatic treatment or less desirably, minimally effective (or toxic) therapy where it is deemed that a no-treatment group is unacceptable. Such a study would determine if (*a*) there is an effect on survival independent of prognostic factor and (*b*) the toxicity achieved by the combination chemotherapy regimen is worth the change in quality of survival as well as in overall survival.

EVALUATION OF RESPONSE

As has been noted above, a number of combination chemotherapy regimens have been identified with response rates ranging from $< 10\%$ to $> 40\%$. The majority of these responses are partial responses which by standard definition require a minimum of a 50% reduction in the cross-sectional area of all measurable disease. Such definitions are well-accepted as standard but present a distinct problem in non-small cell lung cancer (16,25). These patients frequently have multiple lesions, involvement of pleural space and bone, and other nonmeasurable parameters. Thus, the appearance of response is frequently left to considerable subjective criteria. To deal with this problem, Eagan et al (25) have proposed criteria for assessing response in evaluable but not quantitatively measurable disease (eg, pleural effusion). To assess the efficacy of such criteria, they compared survival of patients with measurable disease to that of patients with evaluable disease and showed equivalent survival in responders in both groups. This is certainly an interesting and worthwhile approach to this very difficult area but again raises the question of the comparability of the groups with respect to the prognostic factors.

Since, to date, no single study has convincingly shown that partial regressions of non-small cell lung cancer affect survival independent of the prognostic factors and since most studies contain too few patients to do so, perhaps a more useful criterion for measurement in pilot studies may be the achievement of complete response (ie, complete disappearance of all measurable disease and all signs and symptoms of tumor). The documentation of this extent of response would be very important and would require restaging evaluation similar to that for small cell lung cancer and including bronchoscopy. Thus, a complete response would need to be "pathologically" confirmed. If, in fact, one or more combinations are identified with appreciable complete response frequency, survival of patients who achieve complete response will need to be compared to survival of patients with similar prognostic features who do not achieve a complete response in order to determine that the complete response produced a beneficial therapeutic effect. So far, however, most combination chemotherapy regimens have had only a very low complete response rate.

Histologies

Histologic subclassification may play an important role as a prognostic factor. Most current studies utilize the WHO classification and subclassification (26). The influence of the various histologies, especially that of small cell lung cancer, was recognized in the early 1960s by the VALG, which stressed the need for a uniformly accepted classification and the influence of histology on prognosis (27). At present, there are insufficient data on the prognostic impact of the various non-small cell histologies, perhaps because of the overriding effect of the other prognostic factors. However, as treatment improves, it is conceivable that histology will become important, and major differences among the histologies will be identified. It would be important in considering histology to also evaluate intraobserver and interobserver variability.

Prior Therapy

If it cannot be shown that any combination chemotherapy produces a beneficial effect on survival independent of the prognostic factors, then it is warranted to test new combinations, new agents, or new treatments as primary therapy in non-small cell lung cancer. Certainly the search for more effective treatments must be a high priority for investigative

oncology. Many centers have adopted the policy of testing new agents or new combinations in previously untreated patients with non-small cell lung cancer. One of the reasons for emphasizing the use of new agents in previously untreated (with chemotherapy) patients is the possibility that in addition to the known prognostic factors, prior therapy may reduce the responsiveness to chemotherapeutic agents. For example, when cisplatin was used in previously treated patients, there were fewer responses than when cisplatin was used in patients who had not received prior chemotherapy (28–31). Similarly, an appreciable response rate of nearly 30% was found for vindesine in previously untreated patients (32; BE Furnas, SD Williams, L Einhorn, et al, submitted for publication), whereas far fewer responses have been seen in series of previously treated patients (33, 34). Similar effects on response may be seen with other treatments such as radiotherapy, and careful stratification may be necessary in order to avoid false-positive or false-negative evaluations of chemotherapy. Since the use of marginally effective treatments or chemotherapies that do not produce significant survival benefit may obviate the possibility of finding new and effective agents, chemotherapy of non-small cell lung cancer should remain an investigative modality, with no "standard" chemotherapy prior to investigative efforts.

CONCLUSION

A large number of combination chemotherapy regimens have been introduced for the treatment of non-small cell lung cancer. Initial reports of high response rates are frequently followed by reports of much lesser response rates, leading to difficulties in interpretation and pessimism regarding the treatment of this disease. Although it is conceivable that one or more of the currently utilized combination chemotherapies in fact is beneficial to both the quality and duration of survival, it remains to be demonstrated that any combination chemotherapy is superior to no treatment or produces such an effect independent of predetermined prognostic factors. Considering the large number of prognostic factors and the large number of potential stratifications, it appears that studies designed to answer the question regarding the role of a given combination chemotherapy require a large patient accrual and group efforts. Furthermore, to answer such a question, a given combination chemotherapy needs to be compared to no treatment (palliative therapy only). Until such a beneficial effect from combination chemotherapy can be demonstrated, it appears to be particularly useful to test new antitumor agents or new combinations of active agents in previously untreated non-small cell lung cancer in order to get the best assessment of antitumor activity for these new agents or combinations. Perhaps the best index for assessment of benefit in smaller studies may be the achievement of well-documented complete responses. Hopefully, one of the currently available combinations or possibly a new combination of new and active single agents will be identified that will be useful in prolonging both the quality and duration of survival. Such a combination would then be particularly useful as an adjuvant to localized treatment for stage I and II lung cancer. Until such a treatment can be identified, chemotherapy for non-small cell lung cancer should remain an investigative effort.

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VP-16-213 in the Treatment of Stage III and IV Diffuse Lymphocytic Lymphoma of the Large Cell (Histiocytic) Variety: An Interim Report[1,2]

Peter Jacobs,* Helen S. King, Frances Cassidy, David M. Dent, and Tony Harrison[3,4]

SUMMARY

A pilot study has previously demonstrated antitumor activity for the epipodophyllotoxin VP-16-213 in patients with diffuse lymphocytic lymphoma of the large cell (histiocytic) variety. To define this observation further, a prospective randomized trial was undertaken in patients with stage III and IV disease, comparing this agent used alone (group 1, 41 patients) to the response obtained when the same schedule of VP-16-213 was combined with either cyclophosphamide (group 2, 36 patients) or doxorubicin (group 3, 41 patients). The quantity of VP-16-213 given to patients in the three groups was equivalent. Of these 118 patients, three were lost to followup and thus were excluded from further study. Specifically included were 12 patients who died within 1 month of diagnosis while still receiving induction chemotherapy. For the three groups, complete remission rates were 39%, 26%, and 54%, and additional partial remissions were obtained in 20%, 11%, and 10% of the patients, respectively. The lower remission rates obtained in patients receiving cyclophosphamide combined with VP-16-213 are statistically significant ($P < 0.05$), but no explanation for this is evident since the distribution of poor prognostic factors among the three groups was uniform. The higher percent of complete remission and the greater number of patients remaining free from disease in group 3 demonstrate the superiority of the combination of VP-16-213 with the anthracycline antibiotic doxorubicin.

[Cancer Treat Rep 65:987–993, 1981]

Until recently, the lymphoma designated histiocytic (1) was considered to be a refractory and aggressive neoplasm (2). Of 102 patients treated by us between 1965 and 1972, 52% had clinical stage I or II disease. Some of these patients were curable by appropriate radiotherapy, with our relapse-free survival at 5 years being 23%. The remaining 48% of patients in that original series received cyclophosphamide and vincristine; the median survival was < 6 months. A similar experience is reported in patients treated with combinations of cyclophosphamide, vincristine, and prednisone (3) where complete remissions (CRs) are stated to be infrequent and overall survival poor. Nevertheless, once CR has been achieved, this status will be maintained in a high percent of those patients for long periods of time and without maintenance treatment (4,5). Of greater importance is evidence that the CR rate can be improved by the addition of an anthracycline antibiotic to regimens containing cyclophosphamide, vincristine, and prednisone (6–8).

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[3] The University of Cape Town Leukaemia Centre and the Department of Haematology, Groote Schuur Hospital (*P. Jacobs* and *F. Cassidy*); Departments of Radiotherapy (*H. S. King*), Surgery (*D. M. Dent*), and Pathology (*T. Harrison*), Groote Schuur Hospital, Cape Town, Republic of South Africa.

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Reprint requests to: Peter Jacobs, MD, PhD, Department of Haematology, University of Cape Town Medical School, Research Centre, Anzio Rd, Observatory 7925, Cape Town, Republic of South Africa.

Another drug of interest in treating patients with this histology is the epipodophyllotoxin VP-16-213, which was shown to be of clear benefit in a preliminary evaluation including previously treated individuals (9). In an extension of that study a prospective randomized trial was started in 1974 to define the place of VP-16-213 when used as a single agent or in combination with either cyclophosphamide or doxorubicin in managing late-stage patients with diffuse histiocytic lymphoma. We now present an interim report for the first 118 patients from this protocol study.

This trial, in which patients in one arm were treated with the single agent, was begun before results obtained with multiple cytotoxic chemotherapy regimens were reported (6–8).

MATERIALS AND METHODS

Diagnostic Procedures

At presentation the histopathologic diagnosis was established by excision lymph node biopsy classified according to Rappaport (1). Only patients with diffuse lymphocytic lymphoma of the large cell variety (histiocytic) or those with mixed large and small cell type (histiocytic-lymphocytic) were eligible for study.

All patients had a full physical examination, and the extent of the disease was determined by standard hematologic, biochemical, and radiologic studies. The Ann Arbor classification was used but laparotomy was not done since the patients already had stage III or IV disease. Furthermore, it is our experience that the aggressive nature of this disease and its multicentric origin result in frequent recurrence of disease in surgically negative areas.

Patient Groups

The distribution of the 118 patients is shown in table 1.

Some of the patients in the study had received therapy prior to being referred to us. The details of these treatments are presented in table 2.

Chemotherapy

VP-16-213, previously considered to interfere with spindle formation in mitosis, is known to arrest synchronous cell lines during the S or the G_2 phase of the cell cycle (10).

All the patients received 60 mg/m² of VP-16-213 diluted in 150 ml of physiologic saline and infused iv over 15 minutes on 5 consecutive days. The 500 mg/m² of cyclophosphamide given to group 2 or the 40 mg/m² of doxorubicin given to group 3 were given as an additional slow iv injection on Day 5. Patients then rested for 9 days, and this 2-week cycle was repeated three times in a 6-week period that was designated induction. Patients in group 1 who showed no response were randomized into one of the other two treatment arms. Failure to respond in either group 2 or 3 called for crossover between these two arms.

Responding patients entered a maintenance program in which they received 100 mg of VP-16-213 twice a week as an oral capsule for 3 weeks. In the fourth week, a 5-day course of their respective iv induction regimen was again given.

All patients received continuous maintenance treatment for 6–8 months, after which therapy was discontinued and patients returned regularly for routine clinical followup.

TABLE 1.—Distribution of 118 patients into 3 treatment groups

Group	No. of patients	Drug regimen	No. of patients with—		No. of patients in each stage	
			Mixed cell type	Large cell type		
1	41	VP–16–213 alone	7	34	IV	29
					III	10
					II E*	1
					II SMS*	1
2	36	VP–16–213 and cyclophosphamide on Day 5	5	31	IV	22
					III	12
					II E*	1
					II*	1
3	41	VP–16–213 and doxorubicin on Day 5	7	34	IV	26
					III	14
					II E*	1

*Included as stage IV in view of the high disease bulk, extranodal extension, and the presence of superior mediastinal syndrome.

TABLE 2.—Previous therapy among patients in present study

Group	No. of patients given previous— Radiotherapy	Radiotherapy and chemotherapy*		Chemotherapy		Total No. of patients
1	4	MOPP and bleomycin	1	Cyclophosphamide and vincristine	2	10
				Chlorambucil	1	
				Bleomycin	1	
				Mechlorethamine	1	
2	5	MVOPP	1	MOPP	1	10
		Chlorambucil, bleomycin, and vincristine	1	Chlorambucil and prednisone	1	
		Chlorambucil	1			
3	6	Mechlorethamine	1	Cyclophosphamide	1	10
		Cyclophosphamide and vincristine	1			
		Chlorambucil, vincristine, and cyclophosphamide	1			
Total	15		7		8	30

*MOPP = mechlorethamine, vincristine, prednisone, and procarbazine. MVOPP = mechlorethamine, vinblastine, vincristine, prednisone, and procarbazine.

RESULTS

Exclusions

Of the 118 randomized patients, three were lost to followup and were excluded from further analysis. Included were 12 patients who died within 1 month of diagnosis while receiving their initial chemotherapy: these were the early deaths.

Response to Chemotherapy

In the 115 patients suitable for analysis, antitumor response was defined according to the criteria of the Eastern Cooperative Oncology Group (ECOG) (11). The CR rate for all patients was 40% (table 3). Further analysis showed no statistically significant difference between groups 1 and 3, but the lower remission rate in group 2 was inferior to both of these ($P < 0.05$). It would, therefore, be unethical to continue admitting patients to group 2, and the study has been closed. The median survival of patients achieving CR, irrespective of the form of chemotherapy, has not yet been reached.

At the present time the median duration of followup is 29 months (range, 6–75 months).

Further analysis of the patients achieving CR has been undertaken (table 4). The number of early relapses, defined as the recurrence of disease while receiving maintenance cytotoxic chemotherapy, is substantial in each of the three groups. One possible explanation is that the ECOG criteria used for defining CR may not have been sensitive enough to demonstrate microscopic residual disease. Restaging at the end of the induction chemotherapy was done according to the same criteria as those used when the patients were entered into the study, but lymphangiography was not repeated.

TABLE 3.—Results of chemotherapy*

Group	CR	Median duration of CR	PR	Failed therapy	Early death	Lost to followup
1	16 (39%)	14 mos	8 (20%)	14 (34%)	3 (7%)	0
2	9 (26%)	17 mos	4 (11%)	17 (49%)	5 (14%)	1
3	21 (54%)	11 mos	4 (10%)	10 (26%)	4 (10%)	2
Total	46 (40%)		16 (14%)	41 (36%)	12 (10%)	3

*Unless specified otherwise, values = No. of patients.

TABLE 4.—Analysis of patients achieving CR

	Group		
	1	2	3
Total CRs	16*	9	21
Early relapses (during maintenance therapy)	6	2	10
CRs after completion of therapy	10	7	11
Relapse within 2 yrs from completion of therapy	4	2	3
Relapse after 2 yrs from completion of therapy	2	1	0
Duration of CR after completion of therapy			
< 1 yr	1	2	1
> 1 yr	0	0	2
> 2 yrs	3	2	5

*Values = No. of patients.

Partial response (PR) was achieved in 16 of the 115 evaluable patients (table 3). Median survival was 34 weeks.

Forty-one of the 115 patients failed to respond to chemotherapy (table 3) and had a median survival of 16 weeks. Among the nonresponders there was no difference between groups 1 and 3, but the larger numbers in group 2 were statistically significant ($P < 0.001$). Two types of nonresponders were identified. First, there were those with heavy tumor burden characterized by extensive superficial and deep lymphadenopathy, organ enlargement, or bone marrow invasion by the tumor. The distribution of such patients with poor prognostic signs was uniform among the three groups. Second, a number of patients with light tumor burden inexplicably failed to respond. This latter pattern was unpredictable, and no factors have thus far been recognized that correlate with such primary drug failure.

When comparing CR, PR, and failure to respond to chemotherapy among the groups, a three-way chi square test (12) was used. There was a statistically significant difference ($P < 0.001$) between patients who achieved CR and those with PR or no response.

Prognostic Factors

The dominant factors predictive of poor response to therapy and correlating best with short remission maintenance are the bulk of tumor as assessed by superficial or deep lymphadenopathy, bone marrow invasion, and the demonstration of tumor in the central nervous system or the gastrointestinal tract (13). Although the numbers of patients were small, the distribution of these poor prognostic signs was uniform among the groups and does not appear to explain the inferior results obtained in group 2 where cyclophosphamide was combined with VP-16-213.

Patients having received therapy before referral for protocol study were evenly distributed between the three groups and, to date, this factor does not appear to compromise response to treatment or duration of survival.

The single factor correlating most closely with long disease-free survival is response to initial chemotherapy (fig 1). Nonresponders or partial responders have uniformly poor survival, and it is actuarially predicted that the probability of survival will be superior in group 3 and substantially inferior in group 2 (fig 2).

Interim analysis according to histology and clinical stage of disease has also been done. There is presently no statistically significant difference in response in any of the three groups between patients having large cell histology or those with mixed large and small cell histology. Similarly, there is no statistically significant difference within any of the three groups when response rate is analyzed for patients having stage III or IV disease.

Toxicity

The side effects were limited to alopecia in all of the patients, suggesting that this is due predominantly to VP-16-213. A mild degree of nausea and vomiting was encountered, was easily controlled with antiemetics, and never required discontinuation of therapy.

Myelotoxicity was mild and appeared slightly more often in group 3 than in groups 1 and 2. There was, however, no statistically significant difference. In approximately 10% of the total courses, grade 2 or 3 myelotoxicity (11) was encountered, and the drug dose was appropriately reduced. Grade 4 myelotoxicity was found in < 5% of chemotherapy courses, where granulocyte counts $< 1 \times 10^9$/liter and platelet counts $< 75 \times 10^9$/liter required delay in administration of the next treatment.

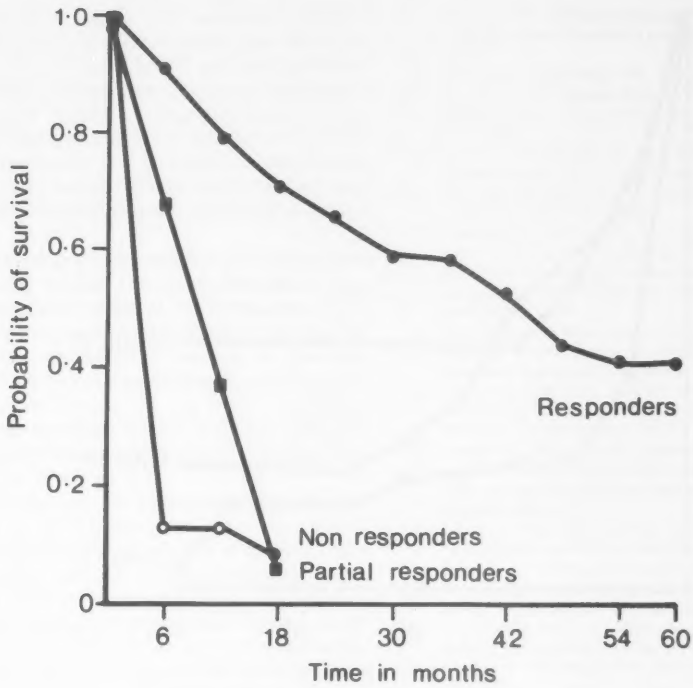

FIGURE 1.—Survival curves for all patients according to response to initial chemotherapy.

DISCUSSION

The clear delineation of the lymphoid nature of these tumors (14,15) was reported after the present study was initiated, and the Rappaport classification (1) has therefore been retained. In staging the patients, the benefits of lymphangiography (16) and staging laparotomy (17) are recognized. However, the high false-negative rate that we have earlier encountered in patients with this histology has led us to classify patients on the basis of clinical staging. Furthermore, since only patients with stage III or IV disease were, by definition, eligible for randomization within the study, the population was defined without recourse to this surgical procedure.

The epipodophyllotoxin VP-16-213 was selected for study because we had earlier demonstrated its activity as a single agent in tumors of this histology (9). That additional benefit might accrue from combining VP-16-213 with cyclophosphamide was suggested by observations in the same study in which this two-drug combination induced a second remission in patients who relapsed after receiving VP-16-213 alone. The third arm combining this agent with doxorubicin was added on the basis of limited personal experience that the anthracycline, either singly or in combination, was active particularly against resistant disease.

After the study had been started and was in progress for some time, reports were published stating that major improvement in treating late stages of diffuse lymphocytic lymphoma of the large cell variety (18) centered on the addition of an anthracycline antibiotic into the COP regimens, giving rise to CHOP (6) or BACOP (7) combinations. It has subsequently also been documented that a disease-free survival extending 2 years beyond the termination of treatment is tantamount to cure (19).

This study confirms that VP-16-213 is an effective agent in treating patients with tumors having this histology. The 39% CR rate obtained by giving VP-16-213 as a single agent is not substantially different from the 48% obtained with the more complex regimens (7). However, the fact that only three of the

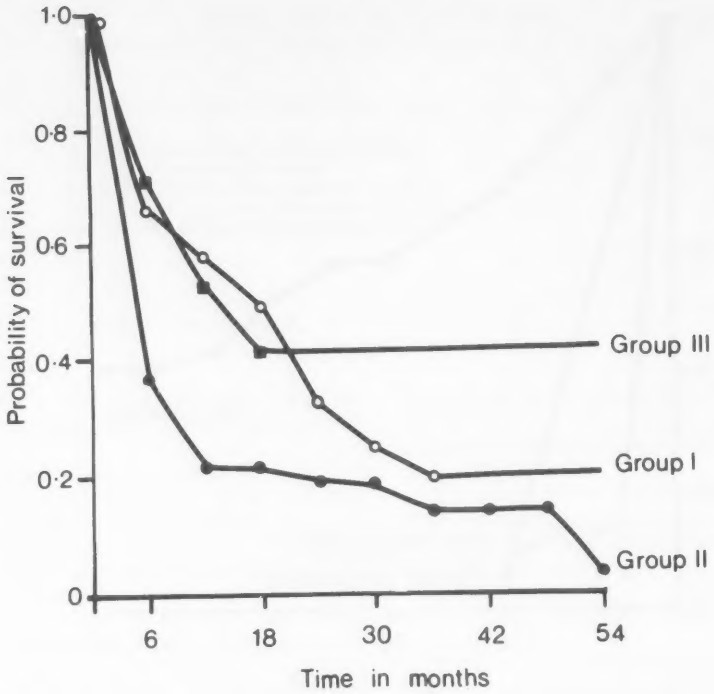

FIGURE 2.—Survival curves for patients receiving VP-16-213 alone (group 1) or combined with either cyclophosphamide (group 2) or doxorubicin (group 3).

16 patients achieving CR have retained this status > 2 years makes it unsuitable for use as a single agent, although it may beneficially be included in the list of second-line drugs or be considered for combination regimens.

The combination of VP-16-213 with cyclophosphamide has given disconcertingly bad results, with the lowest percent of CR. Only four of the nine patients retained this status after treatment, with two still in remission after > 2 years. The explanation for these poor results is obscure. The quantity of drug given was similar in the three groups and there was not a disproportionately high number of patients in group 2 having bad prognostic factors recognizable at presentation. Some pharmacologic interaction may exist in which cyclophosphamide inactivates VP-16-213. Conversely, since the bulk of the VP-16-213 is given before the cyclophosphamide, it is probably more likely that VP-16-213 has some effect on the antitumor activity of cyclophosphamide.

Of greater interest, particularly in view of the results obtained with the anthracycline antibiotics in the combinations, is the higher CR rate found in patients in group 3. Two points deserve comment. First, there are a number of early relapses defined as disease recurrence while the patient is receiving maintenance therapy. The explanation for this has not been established from the present study. Possibilities include failure to have established CR and inadequacy of the regimen. There seems to be no logical reason to believe that the patients had not achieved CR as described by ECOG criteria (11). Whether further lymphangiography should be used to monitor residual retroperitoneal gland enlargement or whether staging laparotomy should be added remain moot points. The adequacy of the combination can only be clearly established by a further randomized prospective trial comparing this regimen directly to CHOP or BACOP, and such a study is now in progress. A point of significance is that, of the patients reaching the end of maintenance

therapy, the only relapses have occurred within 2 years. It remains to be seen whether the other patients have achieved durable CR and are therefore cured. That these patients have achieved prolonged disease-free survival with lesser amounts of cytotoxic agents than are currently employed in four- or five-drug regimens is a theoretic consideration in producing less severe immunosuppression and thereby possibly diminishing the incidence of second malignancies.

The small number of patients being crossed over within the study makes the interpretation of secondary randomization difficult. Nevertheless, it appears that patients receiving doxorubicin after having failed either the single-agent therapy or the combination of VP-16-213 and cyclophosphamide do better, although the results do not reach statistical significance. As in other studies, patients failing to achieve CR constitute a poor prognostic group characterized by short survival.

It is concluded that the epipodophyllotoxin VP-16-213 is active in lymphocytic lymphoma of the large cell (histiocytic) type. It has a limited place as a single agent and its combination with cyclophosphamide detracts from its efficacy. When it is combined with the anthracycline doxorubicin, a significant number of patients survive > 2 years after cessation of all treatment. Determination of the role of this simple two-drug regimen in the treatment of patients bearing this specific tumor awaits the direct comparison of our results to those obtained with the four- and five-drug regimens presently in favor.

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Effective Remission Induction of Refractory Childhood Acute Nonlymphocytic Leukemia by VP-16-213 Plus Azacitidine[1,2,3]

A. Thomas Look,* Gary V. Dahl, David Kalwinsky, Neil Senzer, Clara Mason, and Gaston Rivera[4,5]

SUMMARY

Thirty-eight children and young adults with advanced acute nonlymphocytic leukemia (ANLL) in relapse were treated with VP-16-213 plus azacitidine (5AZ). Each patient had previously received many chemotherapeutic drugs, including anthracyclines and cytarabine. Initially, 16 patients received a 5-day course of VP-16-213 (100 mg/m²) daily × 3 days and 5AZ (150 mg/m²) daily × 2 days, repeated after 9–16 days. Since this treatment produced marrow hypoplasia and complete remission (CR) in only one of 16 patients, a more intensive regimen was devised: the remaining 22 patients received a course of VP-16-213 (200 mg/m²) daily × 3 days, followed by 5AZ (300 mg/m²) daily × 2 days, repeated after 1–2 days until the bone marrow became hypoplastic. After two to four courses, 18 patients had marrow hypoplasia and ten of these achieved CR. The proportion of patients achieving CR with the higher doses was significantly greater than that with the initial doses ($P \leq 0.05$). The toxicity also increased with the higher doses, with major problems due to prolonged pancytopenia. Supportive therapy was required for severe bleeding and infections. We conclude that the intensive treatment with VP-16-213 plus 5AZ can effectively induce remission in patients with refractory advanced acute nonlymphocytic leukemia.

[Cancer Treat Rep 65:995–999, 1981]

Since the introduction of intensive induction chemotherapy with anthracyclines and cytarabine, complete remission (CR) rates of 60%–80% have been reported for newly diagnosed patients with advanced acute nonlymphocytic leukemia (ANLL) (1). Despite remission maintenance therapy with these and other agents, patients have a median remission duration of only 26–50 weeks (1). Second remissions are difficult to induce after patients relapse during chemotherapy, and these patients inevitably become resistant to known effective agents.

The purpose of this study was to assess the therapeutic value of combining VP-16-213 with azacitidine (5AZ)—two drugs with proven activity in single-agent trials for refractory ANLL (2,3). VP-16-213 is a semisynthetic epipodophyllotoxin derivative that induces premitotic arrest of cell progression in vitro and is most cytotoxic for cells in S and G_2 phases (4,5). It has had demonstrable activity in previously treated patients with ANLL (3,6) and in a recent trial, provided an 8% CR rate for 66 children with ANLL in relapse (7). 5AZ is a pyrimidine nucleotide analog that can become incorporated into nucleic acids and interferes with RNA and DNA synthesis (2). This drug showed activity against ANLL in

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[4]Division of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN.

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*_Reprint requests to:_ A. Thomas Look, MD, Division of Hematology-Oncology, St. Jude Children's Research Hospital, PO Box 318, Memphis, TN 38101.

both adults and children (8–10) and in a large cooperative trial (11), 5AZ alone provided a 14% CR rate for 71 patients with ANLL in relapse.

The current report indicates that these two drugs, given in high doses in an intensive schedule, may induce marrow hypoplasia and subsequent CR in about one half of patients with refractory ANLL.

METHODS

Thirty-eight patients with previously treated ANLL were entered in this study. Their characteristics and previous therapy are listed in table 1. The study was designed for patients who had relapsed during therapy or had failed induction during the St. Jude AML 76 protocol study of newly diagnosed patients with ANLL (13). In accordance with this protocol, patients had received vincristine, daunorubicin, 6-azauridine, and cytarabine for remission induction. Continuation therapy with vincristine, cyclophosphamide, doxorubicin, 6-mercaptopurine, and cytarabine had been given in monthly cycles. Thirty of the 38 patients had been initially treated according to the AML 76 protocol. The remaining eight patients had received alternate treatment including anthracyclines and cytarabine. Before VP-16-213 and 5AZ were given, 18 of 38 patients had also received reinduction therapy with methotrexate followed by asparaginase, as described by Capizzi (14). Two patients had failed to respond to 5AZ as a single agent before they were treated with the combination.

Drug Administration

Patients with ANLL in relapse were treated according to one of two regimens. The first 16 patients, treated from February 1978 through January 1979, received successive 5-day courses of VP-16-213 (100 mg/m²) daily for 3 days and 5AZ (150 mg/m²) daily for 2 days. One half of the patients received the three doses of VP-16-213 before the two doses of 5AZ, and one half were given 5AZ for 2 days followed by VP-16-213 for 3 days. Both groups received the same dose of each drug per course, which was repeated after 9–16 days.

The next 22 patients, entered from February 1979 through April 1980, received a more intensive treatment with successive 5-day courses of VP-16-213 (200 mg/m²) daily for 3 days followed by 5AZ (300 mg/m²) daily for 2 days. The interval between courses was shortened to 1–2 days.

With either schedule, courses were repeated until marrow hypoplasia or progressive leukemia was documented. When marrow hypoplasia was observed, chemotherapy was stopped, and bone marrow aspirates were obtained each week to monitor marrow recovery. If leukemic blast cells rather than normal marrow cells returned, two or three additional courses of chemotherapy were given.

TABLE 1.—Patient characteristics

Characteristic*	Initial regimen	Intensive regimen
Total No. of patients	16	22
Males	10	11
Females	6	11
Age in yrs		
Median	13	12
Range	2–19	1–19
ANLL type†		
Myelocytic (M1,M2)	12	12
Progranulocytic (M3)	1	1
Myelomonocytic (M4)	2	5
Monocytic (M5)	1	4
Treatment history		
Induction failure on primary therapy	4	4
First relapse	8	18
2 or more relapses	3	0
AML as second cancer	1	0

*Unless specified otherwise, values = No. of patients.
†Classified according to the FAB Co-operative Group (12).

Criteria for Response

Marrow hypoplasia was defined as hypocellularity of the marrow aspirate with < 5% total blast cells and no identifiable malignant blast cells on two Wright-stained smears. The criteria for CR were findings of a cellular marrow with normal hematopoiesis, < 5% blast cells with no definite leukemic blast cells, and normal blood cell counts.

Supportive Care

Patients were not hospitalized unless treated for infection or active bleeding. Iv gentamicin and oxacillin were used empirically for fever with neutropenia. Packed rbc transfusions were given to maintain a hemoglobin level of 8–10 g/100 ml, and platelet concentrates were used for bleeding associated with thrombocytopenia. Wbcs obtained by continuous flow separation were transfused for documented gram-negative infections that did not respond to antibiotics alone. Total parenteral nutrition was given through a central venous catheter when weight loss exceeded 10% or when serum albumin was < 3.5 g/100 ml in anorectic patients.

RESULTS

The results of therapy with the initial and intensive regimens are compared in table 2. The initial regimen was ineffective, since only one of 16 patients achieved marrow hypoplasia and CR. For patients in the initial treatment, the peripheral blood levels of leukemia blast cells frequently increased during the 1–2 weeks between courses even though these counts decreased during treatment. Progressive disease rather than toxicity was responsible for the therapy failure in 12 of the 16 patients. Therefore, the drug doses were doubled and the interval between courses was decreased to 1–2 days. This intensive regimen was designed to quickly produce marrow hypoplasia and to allow subsequent normal cell recovery. As shown in table 2, this treatment proved effective since 18 of 22 patients had marrow hypoplasia and 10 achieved CR. The proportions of patients with hypoplasia and CR after the intensive treatment were significantly greater than those after the initial treatment [$P < 0.05$, Fisher's test (15)].

The patient on the initial regimen who achieved remission had acute myelocytic leukemia in first relapse and received two courses of therapy. Of the ten patients on the intensive regimen who achieved remission, seven had acute myelocytic, one had acute myelomonocytic, and two had acute monocytic leukemia. To achieve remission, four patients required two courses, four patients required three courses, and two patients required four courses of chemotherapy. Two of these patients had failed primary induction therapy, and the remainder were in first hematologic relapse.

Of the 11 patients who achieved CR, five received no further therapy and remained in remission for 12–29 weeks (median 19), and four received monthly courses of VP-16-213 and 5AZ and remained in remission for 8–27 weeks (median, 10). The remaining two patients had bone marrow transplants during remission: one died of infection 7 weeks later and the other is still in remission 85 weeks after transplant.

TABLE 2.—Efficacy of the 2 therapies

	Initial regimen	Intensive regimen
Total No. of patients	16	22
No. with marrow hypoplasia and—		
CR*	1	10
Leukemic regrowth	0	7
Death in hypoplasia	0	1
Subtotal	1	18
No. without response	12	2
No. who died during induction	3	2

*The proportion of patients who achieved CR and marrow hypoplasia was significantly greater for the intensive regimen ($P < 0.05$).

Toxicity

The major toxic effect produced by the two regimens was prolonged pancytopenia that predisposed to infection and bleeding. With the initial schedule, neutropenia (< 500 cells/mm³) and thrombocytopenia (< 30,000 cells/mm³ or < 2 cells/oil immersion field) each developed in 15 of 16 patients. With the more intensive regimen, all patients had thrombocytopenia and neutropenia when therapy started or within the ensuing 13 days. The duration of pancytopenia was evaluated for patients who achieved CR with the intensive regimen. As shown in table 3, the median interval between the start of therapy and the documentation of CR was 45 days. Neutropenia and thrombocytopenia persisted through most of this period (median durations were 36 and 38 days, respectively).

Aggressive supportive care was necessary, particularly for the 22 patients on the intensive regimen. Twenty of these patients required hospitalization for treatment of fever and neutropenia. The median duration of hospitalization was 21 days (range, 5–103). Five patients received wbc transfusions, and all patients required platelet and rbc transfusions. Total parenteral nutrition was given to six patients. Despite this care, four patients died of hemorrhage associated with thrombocytopenia. Although infections were frequent, none were fatal.

Other toxic effects in all patients included alopecia and moderate to severe vomiting often accompanied by diarrhea after 5AZ infusions. Liver toxicity had been noted during single-agent trials with 5AZ (2). Previous abnormalities of liver function in three patients were exacerbated by VP-16-213 and 5AZ and contributed to the deaths of two of these patients. Three patients on the intensive regimen had reversible renal tubular dysfunction with hypokalemia, hyponatremia, hypomagnesemia, and hypo-

TABLE 3.—Hematologic recovery of 10 patients who achieved CR after intensive therapy

	Median No. of days (range)
Interval from start of therapy to CR	45 (27–84)
Interval from end of therapy to CR	28 (14–49)
Duration of neutropenia (< 500 cells/mm³)	36 (22–67)
Duration of thrombocytopenia (< 30,000 cells/mm³)	38 (11–59)

calcemia, as described for patients who received 5AZ alone (16).

DISCUSSION

The intensive schedule of VP-16-213 and 5AZ in combination is effective for patients with ANLL who fail initial induction therapy or relapse during primary therapy that includes anthracyclines and cytarabine. This regimen was designed to produce marrow hypoplasia rapidly, with only 1–2 days between successive courses, and was superior to the less intensive regimen. Marrow hypoplasia, an objective measure of the effect of chemotherapy on ANLL (17), was evident in a significantly higher proportion of patients who received the intensive therapy (18 of 22 patients with intensive treatment vs one of 16 patients without, $P < 0.05$). In ten of these 18 patients with marrow hypoplasia, the reduction of leukemia cell numbers was sufficient to allow complete recovery of normal marrow function.

The two drugs were combined because they showed promise as single agents in pediatric patients assessed by Karon et al (9), Rivera et al (6), and Chard et al (7). Based on the CR rate achieved with the intensive schedule of VP-16-213 and 5AZ (ten of 22 patients), this combination appears to be one of the best alternatives available for ANLL that is refractory to primary therapy.

The decision to give this intensive combination chemotherapy to an individual patient at relapse depends on the specific drugs and drug regimens used for primary therapy. For example, re-treatment with the same drugs used for first remission induction appears most effective for patients who received no maintenance chemotherapy during initial treatment (18). It is therefore the other patients who fail induction or relapse despite intensive maintenance therapy who should receive the intensive schedule of VP-16-213 and 5AZ, or new drugs like AMSA (19–21).

Since the candidate for VP-16-213 and 5AZ will have shown primary drug resistance during therapy, the success of reinducing remissions may heavily depend on the intensity of the drug regimen. Indeed, among the causes of induction failure classified by Preisler (22), primary drug resistance was the main reason why the initial regimen was ineffective and accounted for 12 of 15 failures. However, when the drug doses were increased, primary drug resistance could be overcome since only two of the 12 failures on the intensive schedule were due to primary resistance. Further support for the importance of combining these drugs in high doses comes from the study of Van Echo and co-workers (23). Their treatment for ANLL in relapse consisted of courses of 5AZ (150 mg/m^2) and VP-16-213 (75 mg/m^2) given daily for 5 days. The 14 patients treated according to this schedule did not achieve remission.

The major complication of our intensive therapy with VP-16-213 and 5AZ was prolonged pancytopenia. The median time to achieve CR was longer than that reported for previously untreated patients who received an anthracycline and cytarabine. For example, Preisler et al (24) reported that one half of their patients, treated with doxorubicin for 3 days and cytarabine for 10 days, were in remission by Day 33. By comparison, the median period until CR in our intensive schedule was 45 days. Part of this delay was due to the increased time to deliver two to four courses of VP-16-213 and 5AZ, but there was also an increased time for marrow recovery after hypoplasia was documented and chemotherapy was stopped. With VP-16-213 and 5AZ the median delay of marrow recovery was 28 days after chemotherapy was stopped, compared to 19 days after a single course of doxorubicin and cytarabine (24). It is not surprising that the duration of marrow hypoplasia and pancytopenia was longer for our patients with refractory disease who previously received intensive chemotherapy.

Despite recent improvement in the initial remission rate for ANLL, most patients eventually relapse during continuation therapy (1). Therefore, it is important to develop more effective drug combinations that can be used earlier in therapy to prevent relapse. In this report, an intensive therapy with VP-16-213 and 5AZ has shown promising activity against refractory ANLL and thus has potential for use during initial therapy.

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Pharmacologic Antidotes to Experimental Doxorubicin Skin Toxicity: A Suggested Role for Beta-Adrenergic Compounds[1,2]

Robert T. Dorr* and David S. Alberts[3,4]

SUMMARY

Doxorubicin (ADM) skin toxicity is a serious complication of inadvertent perivenous drug infiltrations. In an attempt to identify possible antidotes, nine diverse pharmacologic agents were injected intradermally into the hair-free dorsum of BALB/c mice following an intradermal ADM dose of either 0.05 or 0.5 mg. Seven of the compounds were ineffective in reducing ADM-induced ulceration; the compounds included lidocaine, cimetidine, diphenhydramine, sodium heparin, hyaluronidase, *N*-acetylcysteine, and α-tocopherol. The latter five compounds actually increased ulceration induced by ADM (0.5 mg), especially *N*-acetylcysteine, which tripled the total toxic effect. Two opposing beta-adrenergic compounds, the antagonist propranolol and the agonist isoproterenol, reduced skin ulceration resulting from experimental treatment with intradermal ADM. A role for the beta-adrenergic receptor in mediating ADM-induced skin ulceration is suggested.

[Cancer Treat Rep 65:1001–1006, 1981]

The anticipated toxic effects of doxorubicin (ADM) in man include dose-limiting myelosuppression, cardiomyopathy, and alopecia. Local reactions at the injection site can apparently involve two clinical syndromes: (*a*) local hypersensitivity, or "flare" reactions, with erythema and, occasionally, severe pruritus, pain, and/or tenderness (1,2), and (*b*) severe, protracted skin ulceration when inadvertent perivenous infiltration occurs during administration (3–5). The incidence of this latter, more serious toxic effect varies from 4%–6% (6,7) to 0.45% in approximately 2000 patients reviewed by Laughlin et al (8).

Clinically, ADM infiltration can produce slowly progressive erythema and blistering. At 4–5 days after infiltration, lesions progress to clearly demarcated wounds, eventually forming a dark central eschar (3,4). In addition, these lesions characteristically deepen and expand laterally for several months. They may become infected, and separation of necrotic tissue (sloughing) can occur. Large ulcers do not regranulate well and healing is often prolonged. The typical indolent course has been compared to that of radiation-induced skin lesions.

The clinical management of an ADM infiltration is empirical, involving (*a*) immediate treatment after infiltration in an attempt to prevent subsequent ulceration and (*b*) management of necrosis once it has developed. For large evolving lesions, early and wide surgical debridement (beyond the margin of the ulcer), followed by skin grafting or flap coverage, is recommended (8,9). Several local antidotal maneuvers have been recommended, including the infiltration of corticosteroids (3,4) or corticosteroids plus sodium bicarbonate (10), local dilution with saline infiltration, and combined chemical-enzymatic debridement with streptokinase-streptodornase, followed by sodium hypochlorite and dextranomer

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[3]Cancer Center Division, Colleges of Medicine and Pharmacy, The University of Arizona Health Science Center, Tucson.

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**Reprint requests to:* Robert T. Dorr, MS, RPh, Cancer Center Division, The University of Arizona, Arizona Health Science Center, Tucson, AZ 85724.

(11). All of these reports, however, involve only clinical observations and thus lack crucial controls and quantitation of extravasation and lesion size. Several recent reports using experimental animal systems have sought to quantitate the pathology of ADM-induced lesions (12) and to evaluate potential pharmacologic antidotes (13). Previous intradermal ADM studies in the mouse (14) and in the rat (12) have described dose-dependent ADM-induced skin lesions following intradermal injection. Using the mouse skin system, we previously demonstrated the limited effectiveness of low doses of local corticosteroids as antidotes and the ineffectiveness of large doses of sodium bicarbonate and glucocorticosteroids (15). In this report, we describe further studies of diverse pharmacologic interventions among which only beta-adrenergic compounds were significantly effective in reducing ADM skin toxicity.

METHODS

Adult (23–28 g) female BALB/c mice (Jackson Laboratories, Bar Harbor, ME) were used in these studies. The dose-dependent nature of ADM-induced lesions has been described in detail elsewhere (14). The procedure involves preparation of a hair-free site (approximately 3 × 3 cm) on the dorsum of the animal, using one or two applications of the proprietary topical depilatory agent Neet lotion (containing thioglycolic acid, mineral oil, calcium hydroxide, sorbitol, and steryl and cetyl alcohols). One day after hair removal, animals were injected intradermally using a 25-gauge needle (bevel up) containing 0.05 or 0.5 mg of ADM (doxorubicin HCl; Adria Laboratories, Wilmington, DE). All doses were diluted to 0.05 ml, final volume, in 0.9% saline, USP (without preservatives).

Animals were divided into groups of five, were fed a standard laboratory diet ad libitum, and were caged together for the duration of the experiment. Control injections were administered for each antidote tested.

Potential antidotes were injected intradermally, just proximal to the ADM injection site, immediately after the intradermal ADM injection. For all injections, the needle was tunneled intradermally for several millimeters to prevent leakage from the intradermal injection blebs.

The following drugs were tested as antidotes in this study: 7.5 mg of cimetidine (Smith Kline & French Laboratories, Philadelphia, PA), 0.5 mg of diphenhydramine HCl, USP (Benadryl; Parke, Davis & Co, Detroit, MI), 50 units of sodium heparin, USP (Riker Laboratories, Northridge, CA), 7.5 units of hyaluronidase (Wydase; Wyeth Laboratories, Philadelphia, PA), 1.0 mg of lidocaine HCl (Astra Pharmaceutical Products, Inc, Worcester, MA), 10 mg of *N*-acetylcysteine (NAC) (Mucomyst; Mead Johnson Laboratories, Evanston, IL), 10 mg of α-tocopherol as *dl*-α-tocopheryl acetate (Robinson Laboratories, Inc, San Francisco, CA), 10 μg of isoproterenol HCl (Winthrop Laboratories, New York, NY), and 100 μg of propranolol HCl (Inderal; Ayerst Laboratories, New York, NY).

Skin lesions were measured daily by a single observer, with cages identified only by number until the end of the study. Toxicity parameters included the widest perpendicular diameters of concentric areas of induration, erythema, and ulceration. The data were analyzed by computer methods to determine total toxicity in the lesion area (the area under the toxicity-time curve [AUC], in cm^2·days), the peak lesion size (cm^2), and the time to the peak lesion, death, or complete healing. Animals were followed until there was complete resolution to normal of each parameter of toxicity.

Statistical significance was assessed by analysis of variance (ANOVA), with a subsequent Student-Newman-Keuls multiple-range test at an overall error level of 0.05.

RESULTS

Table 1 shows the results of the nine pharmacologic interventions on visible ADM-induced skin ulceration. Most of the agents were unable to significantly reduce ADM-induced skin ulceration. A number of the agents significantly increased skin lesions induced by high-dose ADM (0.5 mg), especially the sulfhydryl donor, NAC. This compound more than doubled the peak lesion size and tripled the total lesion AUC. It also significantly extended the duration of toxic effects by approximately 10 days, compared to that in control mice receiving 0.5 mg of ADM and saline. In contrast, NAC alone did not produce visible ulceration in control animals.

Intradermal α-tocopherol significantly increased ulceration after the 0.5-mg dose of ADM. However, the vitamin slightly reduced ulceration after the lower ADM dose (0.05 mg); the magnitude of the effect was similar to that of isoproterenol against low-dose ADM. Neither reduction reached statistical significance (table 1).

Both antihistaminic compounds, the H_2-receptor antagonist cimetidine and the H_1-receptor antagonist diphenhydramine, were ineffective as antidotes. Cimetidine (7.5 mg) combined with ADM (0.5 mg) proved fatal at 6 days to all five animals treated. Large lesions were uniformly present at the time of death but necropsy was not performed. In control animals, neither antihistamine alone produced death or local skin lesions after intradermal injection.

TABLE 1.—Results of nine pharmacologic agents used as antidotes for skin ulceration following treatment with intradermal ADM (0.05 or 0.5 mg)

Treatment	AUC (cm^2 · days)		Peak level (cm^2)		Damage duration (days)	
	0.05 mg	0.5 mg	0.05 mg	0.5 mg	0.05 mg	0.5 mg
Saline (control)	0.203	6.18	0.050	0.560	8.2	27.3
Cimetidine	0.890*	0.793†	0.105	0.358†	17.8	2.5†
Diphenhydramine HCl	0.965*	12.69*	0.121	0.894	14.0	34.0
Sodium heparin	0.330	10.21	0.037	0.640	12.0	31.5
Hyaluronidase	0.511	11.52*	0.065	0.798	10.3	29.8
Lidocaine HCl	0.470	6.56	0.078	0.715	8.3	32.3
NAC	0.471	25.77*	0.101	1.33*	11.0	37.8
α-tocopherol	0.132	11.63*	0.024	0.816	4.0	29.0
Isoproterenol HCl	0.141	2.36*	0.023	0.174*	3.3*	20.8
Propranolol HCl	0.000*	3.83	0.000*	0.483	0*	28.0

*Significant difference determined by Student-Newman-Keuls multiple-range procedure for the 0.05-mg dose level. This indicates a treatment group not contained in the homogeneous subsets of other treatment groups in which the means of the first and last groups differ by less than the critical value for a subset of that size.

†Low toxicity indices are due to the early death (Day 6) of all animals receiving 0.5 mg of ADM plus cimetidine.

Hyaluronidase was also ineffective in reducing ADM-induced skin ulceration and increased the total toxicity (AUC) of high-dose ADM to a magnitude similar to that of diphenhydramine.

Of the compounds tested, only isoproterenol, a $beta_{1,2}$-agonist, and propranolol, a $beta_{1,2}$-antagonist, were effective in significantly reducing ADM-induced skin lesions.

Propranolol HCl completely prevented skin ulceration and also dramatically reduced total induration and erythema associated with doses of 0.05 mg of ADM. This is graphically compared to the results with saline-treated controls in figure 1. Isoproterenol HCl significantly reduced ADM-induced skin ulceration, erythema, and induration for only the higher dose (0.5 mg) of ADM (fig 2).

DISCUSSION

As in our earlier studies using corticosteroids (14,15), isoproterenol and propranolol in this series reduced both the size of ADM-induced skin ulcerations and the duration of toxic effects. The lesions induced by the 0.5-mg intradermal dose of ADM remained relatively resistant to most antidotal interventions of any type, although they were significantly reduced by a single intradermal isoproterenol intervention (table 1).

Bristow et al (16) have reported that the combined blockade of histamine H_1- and H_2-receptors and adrenergic alpha- and beta-receptors totally prevented experimental ADM cardiomyopathy. These experiments were based on observations in dogs that histamine and catechols were consistently elevated by ADM in parallel with the induction of subacute and chronic ADM toxic effects. In our studies, neither histamine antagonist reduced ADM-induced ulceration.

Sodium heparin was used to test whether a drug known to be incompatible with ADM (17) would complex with free ADM and thereby reduce toxicity. Heparin proved to be ineffective and actually increased skin toxicity of high-dose ADM. Hyaluronidase was similarly ineffective and toxic, thus discounting two rationales for its inclusion: diluting the local intradermal concentration of ADM and augmenting transport of metabolic substrates to a lesion area (18). Lidocaine, which was included to evaluate the role of membrane stabilization in the treatment of ADM skin toxicity, was also ineffective. Both lidocaine and hyaluronidase were also ineffective as antidotes for ADM-induced ulceration in the experimental mouse study of Cohen (13).

The rationale for testing α-tocopherol and NAC was based on the reports that ADM produces free radicals (20). The redox cycling of this quinone compound is thought to cause ADM-induced membrane lipid peroxidation, especially in myocardial mitochondria (21). Thus, Myers et al (22,23) reported a significant reduction in ADM cardiotoxicity and drug-induced lethality in mice pretreated with 55 units of the lipid-soluble antioxidant, free α-tocopherol.

Freeman et al (24) have also recently described enhanced ADM antitumor effects without an increase in toxic effects in mice given cysteamine or NAC (24). These compounds could act as sulfhydryl donors, replenishing depleted cardiac stores of reduced glutathione. A depression in reduced glutathione (the primary intracellular sulfhydryl-containing free radical regulator) is reportedly caused by ADM (25). In the present report, however, neither high-dose α-tocopherol (10 mg; equivalent to

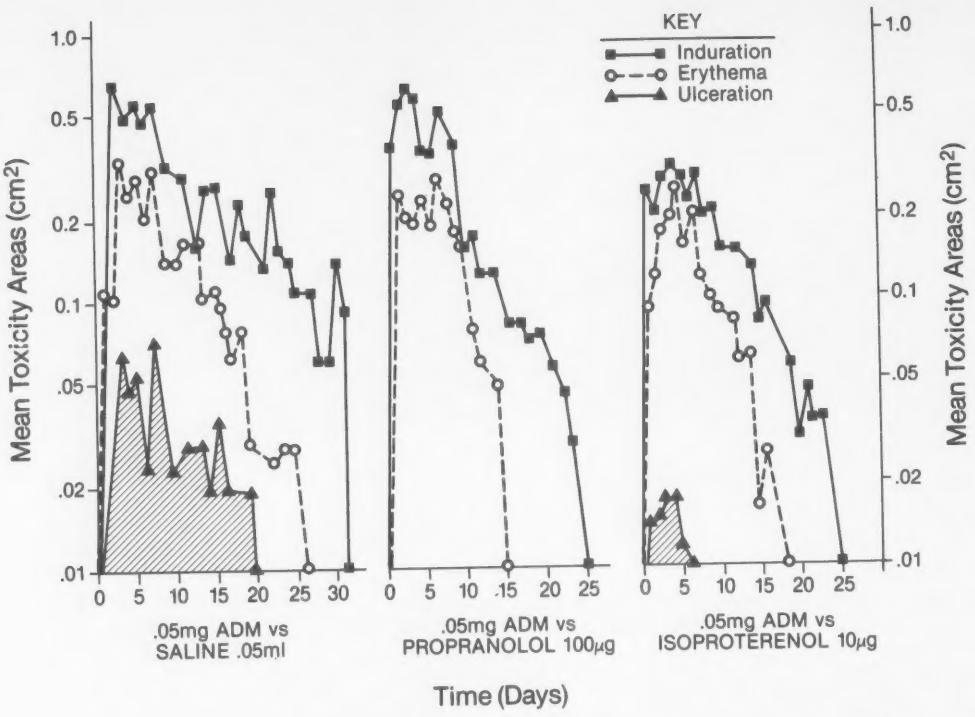

FIGURE 1.—Local skin toxicity following a 0.05-mg dose of intradermal ADM. Hatched areas = the total area of ulcerative toxicity (cm^2·days).

29,000 units) nor NAC (10 mg) proved effective as local antidotes to ADM-induced skin ulceration. NAC more than tripled total ADM skin toxicity (AUC) and doubled peak lesion areas.

Thus, from a diverse group of pharmacologic agents, two classes of compounds have emerged as effective local antidotes against ADM-induced skin ulceration in the mouse. These include corticosteroids at low doses (2.5 mg of hydrocortisone) (15) and two beta-adrenergic compounds, the nonselective $beta_1$- and $beta_2$-antagonist propranolol, and isoproterenol, a $beta_1$- and $beta_2$-agonist. The list of ineffective local compounds now includes large doses of corticosteroids and sodium bicarbonate (15), the histamine antagonists cimetidine and diphenhydramine, heparin, hyaluronidase, lidocaine, the free radical scavenger NAC, and the lipid-soluble antioxidant α-tocopherol. More recently, however, intradermal sodium bicarbonate has been shown to be somewhat effective in an intradermal ADM rat skin model (26).

At this time, the mechanism for the efficacy of the beta-adrenergic compounds seen in this animal model is only speculative. Inasmuch as both compounds produced relatively similar protection from ADM-induced skin ulceration, a mechanism involving alteration of local blood flow is not conceivable since opposite vascular effects would be produced by the two compounds. Beets and Paul (27) have shown that isoproterenol has an effect on vasculature to block inflammatory-mediator-induced increases in the exudation of plasma protein in rodent skin. This effect was distinct from the modest increase in cutaneous blood flow caused by the unopposed beta-agonist injected into the skin. Because the two active compounds in this study are nonselective for $beta_1$- or $beta_2$-receptors, differentiation of a specific receptor interaction is not possible from these data. However, since both compounds were effective in blocking ADM-induced ulceration, a toxic interaction of ADM with beta-receptors in the skin is suggested. The presence of a beta-adrenergic receptor system in mammalian epidermis is well-established (28–30).

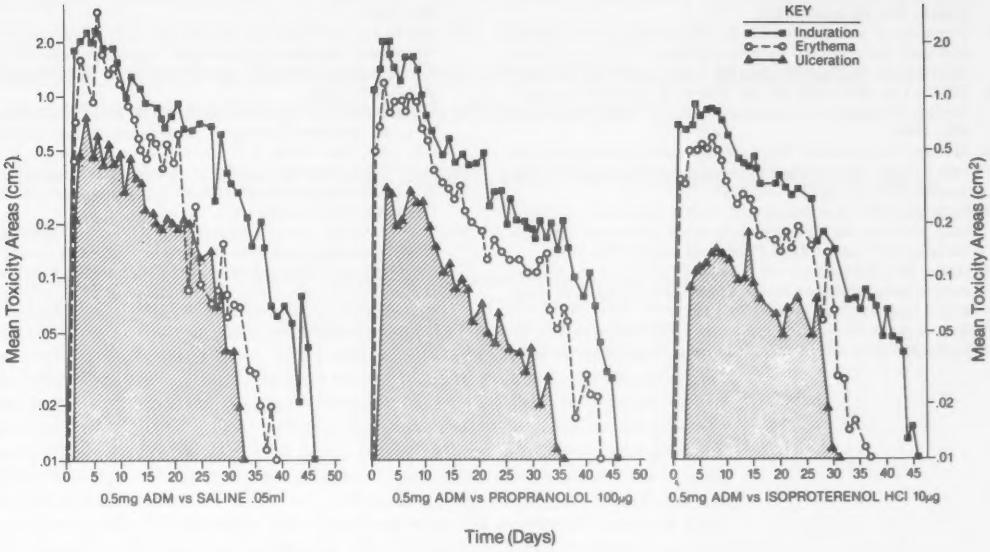

FIGURE 2.—Local skin toxicity following a 0.5-mg dose of intradermal ADM. Hatched areas = the total area of ulcerative toxicity (cm^2·days).

Subsequent work using this mouse model has aimed at more specific modulation of ADM-induced skin ulceration, using different dose schedules of more selective beta-adrenergic compounds (31). These studies may ultimately allow meaningful clinical recommendations to be made for the pharmacologic management of ADM skin infiltrations.

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Factors Affecting Survival of Patients With Acute Myelocytic Leukemia Presenting With High Wbc Counts[1,2,3]

William P. Vaughan,* Allyn W. Kimball, Judith E. Karp, Leon H. Dragon, and Philip J. Burke[4]

SUMMARY

Eighteen patients referred to The Johns Hopkins Oncology Center for treatment of acute myelocytic leukemia during a 2-year period had pretreatment wbc counts > 50,000/μl. Despite immediate admission, aggressive supportive care, and rapid institution of chemotherapy, six patients died from complications of the high wbc count at presentation. The patients who died had significantly higher initial wbc counts, lower platelet counts, and lower arterial oxygen saturation; they were older, more likely to be female, and more likely to have abnormal serum urea nitrogen than the 12 patients who survived. Five of the six patients who died had experienced a delay of > 48 hours from diagnosis to referral, and only one of the 12 patients who survived was not promptly referred for treatment at the time of diagnosis ($P = 0.004$). A mathematical model, the logit of the fraction of the initial wbc count versus the logarithm of time after starting therapy, was used to describe the response of the wbc count to chemotherapy. This logit analysis reduced the data for individual patients to a linear function, permitting statistical description of the population as a whole. This statistical description makes possible the comparison of different approaches to lowering the wbc count in such patients and the correlation of rate of response of wbc count with the probability of achieving remission and with duration of remission.

[Cancer Treat Rep 65:1007–1013, 1981]

Patients with acute myelocytic leukemia (AML) who present with markedly elevated circulating blast cell counts are at risk of early death from endarterial and capillary bed sludging and proliferation of leukemic cells, with tissue invasion and hemorrhage (leukostasis) (1). A number of approaches to the short-term management of these patients have been proposed (2–4), but rapid lowering of the circulating blast cell count seems a critical first step in the prevention of this early mortality. Hydroxyurea, a rapidly acting, cell-cycle-specific chemotherapeutic agent, has most often been used for this purpose (5,6), but the drug is administered orally and is not a very effective agent for induction of remission in AML (7,8). We have previously reported that rapid reduction of the circulating blast cell counts in these patients is most easily accomplished by promptly beginning remission induction chemotherapy with the more effective agents currently available (9). In our institution, this therapy consists of an infusion of high-dose cytarabine (ara-C) and daunorubicin (DRN) (10). Despite this therapy and aggressive supportive care, however, many of these patients still die early in their hospital course from infectious, hemorrhagic, or organ system complications of the high blast cell count at presentation.

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[4]The Johns Hopkins Oncology Center, Baltimore, MD.

*_Reprint requests to:_ William P. Vaughan, MD, The Johns Hopkins Oncology Center, Rm 3–109, 600 N Wolfe St, Baltimore, MD 21205.

This retrospective study was undertaken to help develop better management strategies by better defining the population of patients with AML who present with high circulating blast cell counts and who are at greatest risk of early death. A mathematical analysis of the serial leukemic cell count as a function of time after therapy was undertaken in these patients, and a mathematical model for the effect of chemotherapy on the circulating leukemic cell count was developed to permit statistical analysis of the data.

PATIENTS AND METHODS

During a 2-year period beginning in October 1976, 18 patients with previously untreated AML and > 50,000 blast cells/μl of peripheral blood were referred to The Johns Hopkins Oncology Center. All patients received a rapid diagnostic workup and, within 4 hours of admission, began receiving chemotherapy consisting of timed sequential high-dose ara-C and DRN, the extant protocol chemotherapy for attempting induction of remission at that time. In this regimen, an initial 3-day ara-C infusion was given at a dosage of 28 mg/m²/hour, and a 40-mg/m² dose of DRN was given initially and then daily for a total of three doses. A second ara-C infusion of the same dose and duration was given beginning on Day 8 at the time when the tumor cell proliferative fraction was predicted to be maximally increased. All patients received vigorous supportive care including ventilatory assistance, if necessary. When there was clinical evidence of bleeding, multiple-unit platelet transfusions were given as often as available up to every 6 hours. Rbc transfusions were given only if the total packed-cell volume decreased to < 25%.

Total and differential wbc counts were always determined prior to therapy, but serial differential counts were not performed since, in every case, at least 80% of the circulating wbcs were leukemic cells. The total wbc count determination was repeated at 8 AM on the day following initiation of chemotherapy, if not earlier, and then at least daily until it decreased to < 1000/μl. Other laboratory studies were repeated as indicated. To determine correlation of potential risk factors with early death, the patients who died early were compared to those who survived the initial period; the Mann-Whitney U test was used for continuous variables, and Fisher's exact test was used for dichotomous variables.

The irregular frequency and timing of the wbc count determinations, the variable baseline values, and the nonlinearity of wbc count versus time made it impossible to combine the raw data from all the patients for analysis of the effect of therapy on the wbc count for the population as a whole. Consequently, we sought a mathematical model which would describe the pattern of response of individual patients as a linear function ($y = a + bx$). Several models were considered, including wbc count versus time, the logarithm of the count versus time, and the logarithm of the count versus the logarithm of time, but the best linear representation found was the logit[5] of the fraction of the initial wbc count versus the logarithm of time (logit = a + b[log time]). Unweighted logit regression was used to calculate a slope (b) and intercept (a) for each patient, which could then be combined for statistical analysis. The mean and variance of the slopes and intercepts thus describe the population as a whole, permitting the determination of confidence limits, the comparison of the response of subgroups within the population, and the comparison of the response of these patients with any other groups of patients whose response data can be reduced to a linear function.

RESULTS

These 18 patients had the same age range (16–81 years; median, 51) and sex distribution (ten females and eight males) as unselected patients with this disease. When they were admitted, their wbc counts ranged from slightly over 50,000 to 440,000/μl (median, 87,000). Six patients died early in the course of treatment between 2 hours and 6 days (median, 1.5 days) after the start of therapy. Two other patients died several weeks later with refractory leukemia but survived the high wbc count presentation. Ten patients (56%) achieved complete remission (CR) with one cycle of this chemotherapy, a CR rate approximately equal to that achieved by the entire group of patients treated with this regimen (10). However, the median duration of CR for patients with elevated wbc counts at presentation was less than one half the median duration of CR in patients presenting with low wbc counts (fig 1).

To determine risk factors for early death (table 1), the six patients who died early were compared with the 12 who survived their high wbc count presentation. The patients who died early were all women who were significantly older, had higher total wbc counts, lower platelet counts, and lower arterial pO_2, and were more likely to have an abnormal serum urea nitrogen than the patients who survived. Most significantly, the patients who died early were much

[5] Logit = $\log(p/1-p)$, where p is the fraction of the initial wbc count.

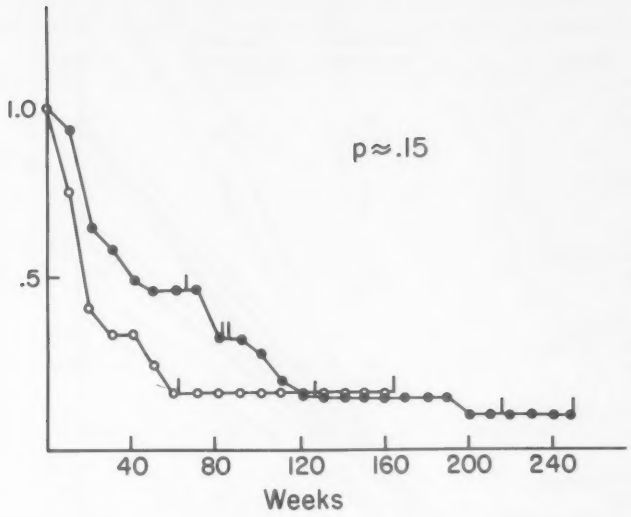

FIGURE 1.—Probability of remaining in CR for 10 patients achieving CR who presented with peripheral leukemic blast cell counts > 50,000/μl (open circles) vs probability of remaining in CR for 34 patients presenting with blast cell counts < 50,000/μl achieving CR with the same therapy during the same time period (closed circles). Neither group received chemotherapy in remission. Median duration of remission was 17 wks for patients with high blast cell counts and 40 wks for those with low blast cell counts ($P \sim 0.15$, log-rank test).

more likely to have spent > 48 hours in another hospital after the diagnosis was established and before referral was initiated.

There was no significant correlation of early death with abnormal uric acid (one of six vs one of 12 patients), potassium (one of six vs four of 12 hypokalemic, none hyperkalemic), alkaline phosphatase (five of five vs 11 of 12), or prothrombin time (three of six vs four of ten < 50%); and patients who died early were not more likely to have clinical evidence of tissue infiltrate (five of six vs 11 of 12) or serious infection on admission (two of six vs six of 12) and had no predominant morphologic subtype of AML (five myeloblastic, one progranulocytic) compared to the patients who survived (six myeloblastic, three monocytic, two myelomonocytic, one undifferentiated). All of the patients who died were white but only two of those who survived were nonwhite. Prior to therapy, 13 patients, including all seven with abnormal prothrombin time and the one patient with progranulocytic morphology, had fibrinogen levels measured, and eight had fibrin split product titers determined. All were normal, so none of these patients received heparin therapy. Three patients developed decreased fibrinogen levels and increased fibrin split product titers during the first 3 days of chemotherapy; however, no patients developed clinically significant diffuse intravascular coagulation (DIC), and the three patients with laboratory evidence of DIC were not among those who had early deaths.

Autopsy showed that all six patients who died early had severe complications of the high wbc count at presentation (table 2) and that one patient each had gram-negative pneumonia, infected pulmonary infarcts, acute myocardial infarction, and disseminated candidiasis, with infected pulmonary emboli. The only patients who did not have factors other than leukostasis contributing to early death were one patient who was comatose and hypotensive on admission and died 4 hours later and one who presented with a subarachnoid hemorrhage but lived 4 days. The two patients who lived > 2 days did so despite pulmonary, renal, and central nervous system failures and only because of extraordinary life-support measures.

The plots of the logit fits for individual patients (fig 2) demonstrate some variation in the slope of

TABLE 1.—Risk factors for early death from high wbc count in AML*

Factor	Early death (6 patients)	Survival (12 patients)	*P* value	Test†
Age (yrs)				
Median	63	34	< 0.01	U
Range	52–77	16–81		
Sex				
Females	6	4	0.01	FE
Males	0	8		
Wbc count (cells × $10^3/\mu l$)				
Median	240	79	< 0.05	U
Range	76–424	52–440		
Platelet count (cells × $10^3/\mu l$)				
Median	34	71	< 0.05	U
Range	20–64	26–265		
Arterial pO_2 (mm Hg)‡				
Median	41	52	< 0.025	U
Range	35–55	40–70		
Serum urea nitrogen (mg/dl)	9–45	5–20		
No. of normal readings	3	12	0.02	FE
No. of abnormal readings	3	0		
Delayed referral§	5	1	0.004	FE

*Race, uric acid, potassium alkaline phosphatase, prothrombin time, morphology, tissue infiltrate, and serious infection were not significant.

†U = Mann-Whitney U test, FE = Fisher's exact test.

‡Specimen was placed in ice at the bedside and transported immediately to the laboratory; test was performed on arrival to minimize oxygen consumption in the syringe.

§Values = No. of patients who had spent > 48 hrs in another hospital after diagnosis and before referral.

TABLE 2.—Causes of death in patients with AML who presented with high wbc counts

Patient No.	Condition on admission	Time before death	Autopsy findings
1	Coma, acidosis	2 hrs	Leukostasis in brain, heart, and lungs
2	Semicoma, fever, dyspnea	24 hrs	Leukostasis in brain and lungs; infected pulmonary infarct
3	Dyspnea, acute myocardial infarct	33 hrs	Leukostasis in heart and lungs
4	Semicoma, pneumonia	40 hrs	Leukostasis in lungs; gram-negative pneumonia
5	Semicoma, dyspnea, bloody spinal fluid	4 days	Leukostasis in brain and lungs; diffuse necrosis in the cerebrum, cerebellum, and brain stem; subarachnoid hemorrhage
6	Semicoma, dyspnea, decubitus ulcers	6 days	Disseminated candidiasis, with infected pulmonary emboli; massive pulmonary hemorrhage

the fits, suggesting variation from patient to patient in the response of the circulating blast cell count to chemotherapy. However, four of the six patients who died early died too early to have the necessary three wbc count determinations to perform this logistic analysis, and the two patients who did not achieve CR are too few for statistical comparison, so any correlation of clinical outcome with rate of wbc count decrease in response to chemotherapy could not be determined. However, the combined

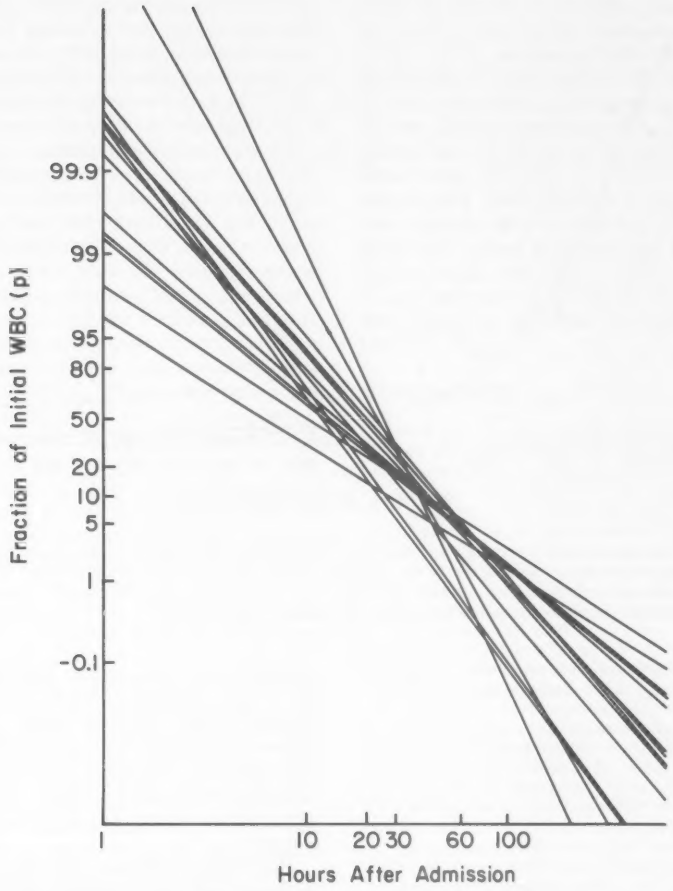

FIGURE 2.—Plots of logit fits of total wbc counts vs log time for 14 patients with AML and presenting wbc count > 50,000/μl who were treated with DRN and high-dose ara-C.

logistic analyses permit analysis of response of peripheral wbc count to this therapy by the population as a whole (fig 3). The mean time to 50% reduction of initial wbc count was 16 hours, and 95% of the patients can be expected to have wbc counts that are decreased to < 50% of the initial value by 24 hours.

Closer examination of the superimposed fits of the individual patients' logits demonstrated a remarkable convergence of the fits at 11% of the initial wbc count at 33 hours. It can be calculated from the data that 95% of the patients achieve reduction to 11% by 48 hours and that 95% of the patients have wbc counts < 26% of the pretreatment value by 33 hours (table 3). Differential wbc counts were done in most patients after 2–3 days of therapy and revealed that most of the residual cells were not leukemic blast cells.

DISCUSSION

Death prior to receiving an adequate trial of chemotherapy remains a major problem in the management of AML, and patients presenting with high circulating blast cell counts are at great risk of early death. Our data demonstrate that even when chemotherapy capable of rapidly lowering the wbc count

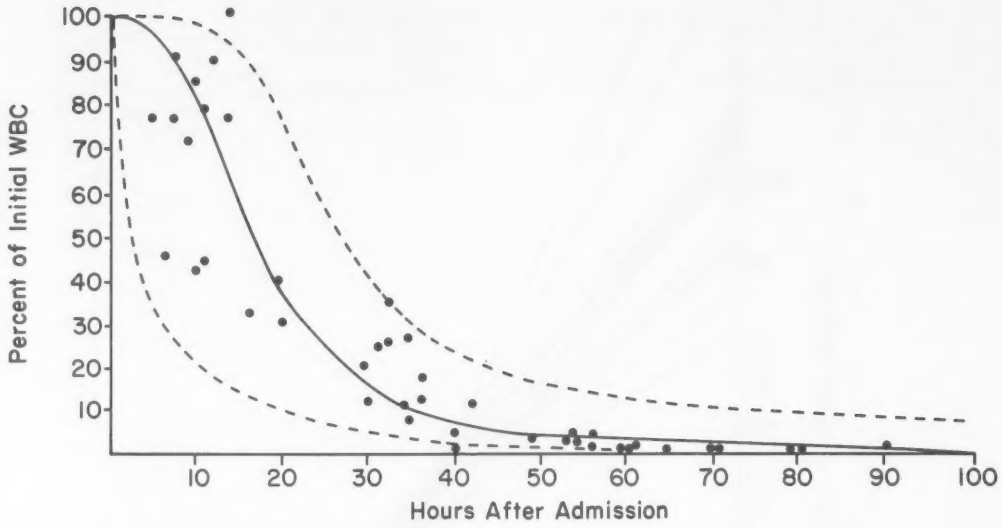

FIGURE 3.—Effect of therapy with DRN and high-dose ara-C on the peripheral wbc count in patients with leukemia presenting with wbc counts > 50,000/μl. Solid line = mean of the % wbc count vs time for the 14 patients analyzed using the logit function; dashed lines = 95% confidence limits for individual patients; and dots = the actual data. 41 of 43 (95.3%) of the actual data points fall within the 95% confidence limits as determined by analysis of variance of the logit fits.

TABLE 3.—Effect of ara-C and DRN treatment for high blast cell count in AML at point of minimum variation among patients*

Patient No.	Time to 88.6% reduction (hrs)	% initial wbc count at 33.4 hrs
1	31.0	9.5
2	36.5	13.5
3	22.2	3.3
4	35.0	14.2
5	34.6	12.2
6	23.4	7.0
7	34.8	12.3
8	42.6	20.9
9	44.2	21.7
10	23.0	3.5
11	36.4	12.9
12	36.8	15.2
13	36.8	14.2
14	39.5	17.3
Mean	33.4	11.4
Calculated 95th percentile	48.0	26.1

*Values are calculated from logit fits.

is given, some patients will still die of complications of the high wbc count at presentation. The poor prognosis associated with most of the factors we identified appears to have a direct relationship to the pathophysiology of the high blast cell count at presentation. The higher the blast cell count and the longer the time it was elevated prior to initiating chemotherapy, the more likely there was to be extensive tissue infiltrate and proliferation, with vascular damage and organ system compromise. If the platelet count was depressed, the hemorrhage that occurred spontaneously or with treatment was more severe.

Subclinical DIC was detected in several of these patients, but the lack of correlation with early death suggests that DIC may not be a risk factor for early death except to the extent that it contributes to thrombocytopenia. Markedly elevated uric acid and severe electrolyte abnormalities were present in several patients but could be successfully managed unless renal function was compromised. Older patients probably did less well as a result of a greater incidence of serious pre-existing medical illness, but the referral of these patients tended to be a slower process as well. The reason that all of the patients who died early were women is obscure, but they all had multiple other risk factors including advanced age and delayed referral.

It appears that some patients are rescued from early death by early referral, immediate chemotherapy, and vigorous supportive care. Since hemorrhage is such a prominent feature of this syndrome and thrombocytopenia is an identified risk factor,

transfusion of large numbers of platelets probably is a very important aspect of this supportive care. Rbc transfusion, on the other hand, increases whole-blood viscosity and may be contraindicated until the high circulating blast cell count is controlled (11,12).

Additional measures used in the management of this syndrome have included leukapheresis and cranial irradiation. Removal of large numbers of cells by pheresis has been shown to be capable of reducing the circulating blast cell count (3,13), but it does not affect the proliferation of cells already lodged in tissue and may complicate the management of the medically unstable patient. Cranial irradiation offers the potential advantage of immediate antiproliferative effects directed toward a critical organ but probably works no faster than adequate doses of iv administered ara-C. These and other approaches to these critically ill patients deserve further study, but the most important element in the management of this syndrome appears to be early referral. Five of the six early deaths occurred in patients whose referral was delayed as little as 48 hours, and three of these patients were comatose and hypotensive at admission or within a few hours of admission.

Even if the patient survives and achieves a CR, however, that CR is likely to be short. This association between high initial wbc count and short remission has also been noted by Keating and colleagues (14). The most logical reason for remissions of shorter duration in patients presenting with high circulating blast cell counts is that the high blast cell count is a manifestation of greater total body burden of malignant cells, resulting in a larger residual tumor burden after treatment. Such patients might be expected to benefit from chemotherapy in remission. Keating's patients did receive maintenance chemotherapy and did have a slightly longer median duration of remission than our patients, but the 75th and 25th percentile figures are similar, and six of the eight patients in our series who have relapsed and were retreated with the same therapy achieved a second remission, so survival may be comparable.

Comparison of the clinical outcome of this syndrome, using different treatment strategies, even between institutions which accept all referred patients, is difficult since the clinical outcome is dependent on the presenting features in addition to the wbc count (15). However, statistically valid comparisons of different approaches to decreasing the wbc count can be made if the data can be reduced to a linear function, using the logit or other mathematical models. In addition, the rate of response of the wbc count to chemotherapy within any given series can be correlated with clinical outcome such as CR rate or duration of CR. The appropriateness of the logit model for reducing our data is confirmed by the observation that 95% of the original data points fall within the 95% confidence limits calculated using the logit fits (fig 3 and table 3). The observation that the point of minimum variance of our response data occurred at 90% reduction in the initial wbc count suggests that time to 90% reduction would be the most useful single point for evaluating response of individual patients to therapy or for comparing different approaches to decreasing the wbc count.

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Comparison of the Therapeutic Response of Patients With Childhood Acute Lymphoblastic Leukemia in Relapse to Vindesine Versus Vincristine in Combination With Prednisone and L-Asparaginase: A Phase III Trial [1,2]

James Anderson, William Krivit, Robert Chilcote, Allan Pyesmany, Ronald Chard, and Denman Hammond [2,3,*]

Writing for the Children's Cancer Study Group

SUMMARY

One hundred and seventy-nine previously treated children with acute lymphoblastic leukemia in relapse considered not "resistant" to vincristine (VCR) were randomly allocated to receive reinduction therapy with either vindesine (VND) or VCR, in combination with prednisone and L-asparaginase. Complete remission rates were 57% for both regimens and were significantly greater for first relapsers (69%) than for subsequent relapsers (43%). No significant difference in response rates by regimen was observed within relapse groups. Patients treated with VND experienced significantly greater hematologic toxicity. These data suggest that there is no advantage to using VND instead of VCR in standard reinduction therapy for childhood acute lymphoblastic leukemia in relapse.

[Cancer Treat Rep 65:1015–1019, 1981]

Although notable advances have been made in the treatment of childhood acute lymphoblastic leukemia (ALL), nearly 50% of the children still relapse, most during the first 3 years of therapy. With current treatment programs, patients with ALL who relapse have little chance for long-term survival (1); thus, new agents and treatment strategies are re-

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[2]This study was conducted by the Children's Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Group Operations Office, University of Southern California, Comprehensive Cancer Center, Los Angeles (*D. Hammond, R. Honour, H. Sather, J. Anderson,* and *J. Weiner,* CA-13539); University of Michigan Medical Center, Ann Arbor (*R. Heyn,* CA-02971); Children's Hospital National Medical Center, George Washington University, Washington, DC (*S. Leikin,* CA-03888); Memorial Sloan-Kettering Cancer Center, Cornell University, New York, NY (*D. R. Miller,* CA-23742); Children's Hospital of Los Angeles, University of Southern California (*G. Higgins,* CA-02649); Babies Hospital, Columbia University, New York, NY (*J. Wolff,* CA-03526); Children's Hospital of Pittsburgh, University of Pittsburgh, PA (*V. Albo,* CA-07439); Children's Orthopedic Hospital and Medical Center, University of Washington, Seattle (*R. Chard,* CA-10382); University of Wisconsin Hospitals, Madison (*N. Shahidi,* CA-05436); University of Minnesota Health Sciences Center, Minneapolis (*M. Nesbit,* CA-07306); Children's Memorial Hospital, Northwestern University, Chicago, IL (*G. Honig,* CA-07431); Strong Memorial Hospital, University of Rochester, NY (*M. Klemperer,* CA-11174); University of British Columbia, Vancouver, Canada (*M. Teasdale,* The Vancouver Foundation); Children's Hospital of Philadelphia, University of Pennsylvania (*A. Evans,* CA-11796); James Whitcomb Riley Hospital, Indiana University, Indianapolis (*R. Baehner,* CA-13809); Harbor General Hospital, University of California at Los Angeles, Torrance (*J. Finklestein,* CA-14560); University of California Medical Center, San Francisco (*A. Albin,* CA-17829); Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH (*S. Gross,* CA-20320); Vanderbilt University School of Medicine, Nashville, TN (*J. Lukens*); University of Texas Health Sciences Center, San Antonio (*T. Williams*); University of California Medical Center, Los Angeles (*S. Feig,* CA-27678); Children's Hospital of Columbus, Ohio State University (*W. Newton,* CA-03750); University of Oregon Health Sciences Center, Portland (*R. Neerhout,* CA-26044); and Children's Hospital Medical Center, University of Cincinnati, OH (*B. Lampkin,* CA-26126).

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**Reprint requests to:* Children's Cancer Study Group, Operations Office, 1721 Griffin Ave, Los Angeles, CA 90031.

quired if these patients are to be successfully retreated. The vinca alkaloid vindesine (VND) has been shown to be an active agent in the treatment of childhood ALL. In a phase II study conducted by the Children's Cancer Study Group (CCSG), three complete and 13 partial remissions were observed when 35 patients who had received prior multiagent chemotherapy were treated with VND (4 mg/m² iv weekly × 3) alone (2). This study prompted the CCSG to initiate a randomized phase III trial comparing VND to vincristine (VCR) in the treatment of ALL in relapse. An assessment of the relative merits of these two agents when utilized in reinduction with prednisone and L-asparaginase is the subject of this report.

MATERIALS AND METHODS

Patients

Patients were eligible for entry in the study if they had been previously treated for ALL and were in relapse as evidenced by a bone marrow aspirate containing > 25% lymphoblast cells. The relapse did not have to be the patient's first, and there was no requirement that all patients had to have received uniform prior therapy.

Induction therapy was determined, in part, by whether a patient was considered "VCR-resistant." Resistance to VCR was defined arbitrarily as the failure to achieve a remission with induction therapy in which VCR was used in combination with other drugs. Patients who relapsed during maintenance therapy with VCR were *not* considered resistant. The results of induction therapy for the VCR-resistant patients have been reported elsewhere (3). Only the induction results for patients not VCR-resistant are reported here. Patients have been grouped for analysis on the basis of the number of bone marrow relapses preceding entry in this study. Group 1 includes all patients known to be in first bone marrow relapse when entered in the study; group 2 includes all patients known to be in a second or subsequent relapse; and group 3 includes those patients for whom the number of bone marrow relapses prior to entry in the study was unknown, and thus a mixture of patients in terms of the number of marrow relapses preceding entry in the study.

Patients were randomized to receive a 28-day course of either VND (4 mg/m² iv weekly × 4) or VCR (1.5 mg/m² iv weekly × 4; maximum single dose, 2.0 mg) together with prednisone (40 mg/m² orally daily) and L-asparaginase (6000 units/m² im, every other day × 3) as reinduction therapy.

Cbcs were usually available for each patient on Days 0, 7, 14, and 21, the prescribed days of vinca alkaloid administration, and on Day 28, the usual end of the induction phase. The data used to assess the relative hematologic toxicity of VND and VCR were the absolute neutrophil and platelet counts recorded on these 5 days, regardless of whether delays in therapy occurred as a result of toxicity. In some cases, if data were unavailable for a particular day, but were available a day or two before or after, the closest available data were used. The number of observations available at each time point varies because some patients did not complete a full induction course and others had data for only some of the prescribed evaluation times.

Response to therapy was evaluated at Day 28. An M_1 marrow (complete bone marrow remission) was defined by a bone marrow aspirate containing 0–5% blast cells; an M_2 marrow was described as containing 6%–25% blast cells; and an M_3 marrow was described as containing > 25% blast cells. Patients treated in the study, but withdrawn before Day 28 because of objective evidence of progressive disease or toxicity or for other reasons related to treatment, were considered induction failures (M_x), as were patients who died during the induction phase.

All patients who received any protocol therapy have been included in this analysis. Three patients who were registered in the study but who died before the initiation of therapy have been excluded. Informed consent for treatment was obtained for all patients in accordance with institutional policies approved by the US Department of Health and Human Services.

RESULTS

Remission Induction

One hundred and eighty-three VCR-nonresistant patients were treated: 91 with the regimen containing VND (regimen 1) and 92 with the regimen containing VCR (regimen 2). Induction results were unknown for four patients (two patients in regimen 1; two in regimen 2). Of the remaining 179 patients, 102 attained a complete remission (57%) (table 1).

Induction results were compared by relapse group, regimen, and by regimen within relapse group. Induction rates differed significantly by relapse group. Complete response rates were: 69% (58 of 84 patients) in group 1; 43% (30 of 69 patients) in group 2 ($P = 0.001$); and 54% (14 of 26 patients) in group 3.

TABLE 1.—Induction results

	M_1	M_2	M_3	M_χ	Died	Total
No. of evaluable patients	102*	23	31	8	15	179
Group 1	58	7	11	3	5	84
Group 2	30	12	18	5	4	69
Group 3	14	4	2		6	26
Regimen 1	51	13	11	3	11	89
Regimen 2	51	10	20	5	4	90
Group 1						
Regimen 1	25	3	4	1	4	37
Regimen 2	33	4	7	2	1	47
Group 2						
Regimen 1	19	7	7	2	3	38
Regimen 2	11	5	11	3	1	31

*Values = No. of patients.

Relative Hematologic Toxicity

Absolute Neutrophil Counts (table 2)

The distribution of absolute neutrophil count (ANC) for patients in either regimen was nearly identical at Day 0 ($P = 0.61$, Wilcoxon test). While both regimens suppressed the number of neutrophils, significantly more hematologic toxicity was experienced by patients treated with VND. At Day 14 (the date when the ANCs for both regimens reached their nadir), the median ANC for patients treated with VND was 260/mm³ and the median ANC for patients treated with VCR was 1122/mm³ ($P = 0.0001$). An ANC > 200/mm³ is thought to be necessary to provide adequate protection against infection (4). At Day 14, nearly one half of the patients treated with VND had an ANC < 200/mm³; less than one quarter of those treated with VCR had an ANC < 200/mm³ ($P = 0.004$). While recovery of the ANC occurred in both regimens, at the end of induction the ANC for patients treated with VCR was much higher than that for patients treated with VND. VCR-treated patients had a median ANC twice that of VND-treated patients ($P = 0.007$) and only 5% of them had an ANC < 200/mm³ at the end of induction, compared to 14% for those receiving VND.

No significant differences in induction rates were observed by regimen, even when the comparisons were made within relapse groups (table 1). The overall induction rate was 57% for both regimens. For patients in group 1, complete response rates were 68% for regimen 1 and 70% for regimen 2 (P

TABLE 2.—Distribution of ANCs by induction regimen

	Day—				
	0	7	14	21	28
			Regimen 1		
ANC by percentile (cells/mm³)					
75%	3024	1815	784	1230	2580
50%	1426	576	260	600	1364
25%	588	162	40	130	500
% of patients with ANC ≤ 200 cells/mm³	9	36	46	30	14
No. of patients	71	64	55	56	57
			Regimen 2		
ANC by percentile (cells/mm³)					
75%	2560	3366	2750	3724	4602
50%	1420	1836	1122	2464	2860
25%	392	480	240	660	1485
% of patients with ANC ≤ 200 cells/mm³	10	16	21	13	5
No. of patients	70	62	67	62	65

= 0.79). For patients in group 2, the complete response rates were 50% for regimen 1 and 35% for regimen 2 (P = 0.22). While the complete response rates for both regimens were comparable, more patients treated with VND died during induction: 11 of 89 patients (12%) treated with VND compared to four of 90 (4%) treated with VCR (P = 0.06).

The effect of the regimens on ANC was also compared for groups 1 and 2 separately. In both groups, patients who were treated with VND experienced a significantly lower ANC. However, the difference in nadir ANC between the two regimens was greater for patients in group 2. At Day 14, median ANCs were: 250/mm^3 for group 1 patients in regimen 1, 648/mm^3 for group 1 patients in regimen 2 (P = 0.01), 380/mm^3 for group 2 patients in regimen 1, and 2184/mm^3 for group 2 patients in regimen 2 (P = 0.001). The myelosuppression caused by VCR appears to be less for patients in a second or subsequent relapse whereas the effect of VND does not appear to be changed substantially.

Platelet Counts

The distribution of platelet counts for patients in regimens 1 and 2 was similar at the beginning of the study. Platelet counts were not adversely affected during induction therapy. In fact, the tendency was for platelet counts to rise during the induction period, with patients treated with VND showing the greater increase. The median platelet count for patients treated with VND was 120,000/mm^3 at the beginning of the study, increasing to 220,000/mm^3 at the end of induction. For patients treated with VCR, the median platelet count at the beginning of the study was 152,000/mm^3, increasing to 175,000/mm^3 at the end of induction.

Other Toxic Effects

A review of patient records showed that severe jaw pain, fevers > 102°F, abdominal pain, and constipation occurred in twice as many patients treated with VND compared to those treated with VCR (22% vs 11%). Minor complications such as loss of tendon reflexes occurred equally in both groups.

Time to Relapse and Survival

Patients achieving either an M_1 or M_2 marrow at the end of induction received maintenance therapy which consisted of the vinca alkaloid given during induction plus cyclophosphamide. Patients were randomized to receive the cyclophosphamide either with, or 24 hours after, the vinca alkaloid. Median time from the start of maintenance therapy to bone marrow relapse was 13 weeks for patients receiving VCR and 11 weeks for patients receiving VND. Ultimate survival for both groups of patients was the same; the median survival was 28 weeks.

DISCUSSION

The vinca alkaloid analog VND has been established as an active agent in the treatment of childhood ALL. Phase II studies have shown that about 30% of patients with ALL considered refractory to standard therapy can be expected to achieve at least a partial remission when treated with VND alone (2).This randomized study was undertaken to assess the relative efficacy and toxicity of VND and VCR when used in combination with prednisone and L-asparaginase in the treatment of childhood ALL in relapse. For patients being treated in first relapse, no difference in induction rates was observed. Complete response rates were: 68% in regimen 1 and 70% in regimen 2. These results compare favorably with other reported second induction rates (5). For patients treated in a subsequent relapse, those receiving VND had a complete response rate of 50% versus 35% for those treated with VCR, but this difference was not statistically significant (P = 0.22).

Significantly increased hematologic toxicity was experienced by patients treated with VND. The higher VND dose per square meter (4 mg/m^2 for VND vs 1.5 mg/m^2 for VCR) was used because it is the "tolerated" dose determined in phase I–II trials (6,7). Neurologic toxicity limits any single dose of VCR to a maximum of 2 mg/m^2. At equivalent doses, neurologic toxicity is not as extreme a problem with VND; thus, no maximum dose need be defined. The higher dose of VND used may have been partially responsible for the increased neutropenia observed. Whether an equivalent therapeutic response could have been observed if VND had been given at the same dose used for VCR is unknown.

An increase in platelet counts was observed in both regimens; however, the effect seemed to be somewhat more pronounced for patients receiving VND. Other investigators have observed these effects of VND on neutrophil and platelet counts (6,8–10).

Vinca alkaloids cause neurotoxicity by slowing fast axoplasmic transport and by binding tubulin (11). Neurotoxicity was experienced by patients in both regimens, but about twice as many patients treated with VND experienced major complications. Significant VND neurotoxicity has also been reported by others (8–10).

Standard reinduction therapy for childhood ALL usually includes VCR as one of the agents, often in conjunction with prednisone and other drugs. Some

patients unresponsive to treatment which has included VCR will respond to induction therapy which includes VND (3). However, the data presented here do not indicate an advantage to using VND over VCR in reinduction therapy for patients not resistant to VCR.

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Pharmacokinetic Evaluation of Cisplatin in Children With Malignant Solid Tumors: A Phase II Study[1,2,3]

Charles B. Pratt,* Ann Hayes, Alexander A. Green, William E. Evans, Neil Senzer, Cathryn B. Howarth, J. Laurence Ransom, and William Crom[4]

SUMMARY

In a phase II trial, cisplatin was given by two randomly assigned dose schedules to 53 children with malignant solid tumors. Both schedules, 30 mg/m²/week and 90 mg/m² every 3 weeks, were associated with drug-related toxic effects involving the renal, hematopoietic, and neuromuscular systems. Pharmacokinetic studies indicated that the initial serum half-life of total platinum was 27.6 minutes, with a terminal half-life of 44.4 hours. Significant antitumor effect was noted in patients with neuroblastoma and malignant germ cell tumors. Use of cisplatin in combination therapy for patients with these diseases is indicated.

[Cancer Treat Rep 65:1021–1026, 1981]

Since 1972, clinical trials with cisplatin (CDDP) have been carried out under the sponsorship of the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD (1). The drug's therapeutic effectiveness has been recognized for the treatment of testicular tumors as well as other solid tumors of adults, including ovarian, bladder, and head and neck tumors (2–5).

The objectives of the following studies were to evaluate the effectiveness of CDDP in children with malignant solid tumors refractory to conventional chemotherapeutic agents, to determine the toxic effects in relation to dose scheduling, and to evaluate the pharmacokinetics of CDDP.

PATIENTS AND METHODS

Between November 1977 and February 1980, 53 patients between the ages of 6 months and 20 years were treated with CDDP by one of two dose schedules. All of the patients met the requirements for admission to this study by virtue of resistance of their tumors to conventional chemotherapeutic agents, the presence of measurable indicators of disease activity, normal renal function, recovery from toxic effects induced by previous agents, and a life expectancy of at least 6 weeks. Informed consent of the patients or their parents was obtained prior to admission to the study.

Ten patients were randomized to receive CDDP iv at a dose of 30 mg/m² once weekly, and 15 were randomized to receive CDDP at a dose of 90 mg/m² iv once every 3 weeks. An additional 28 patients were not randomized but were treated with CDDP at a dose of 90 mg/m² every 3 weeks. Prehydration consisted of mannitol (10 g/m² in 5% glucose and 0.3% saline) given iv in a volume of 500 ml/m² for 2 hours prior to the CDDP infusion. Furosemide (15 mg/m²) was given iv immediately prior to the infusion of the CDDP. The CDDP was mixed with mannitol (10 g/m² in 5% dextrose and 0.3% saline) and given iv in a volume of 1000 ml/m² over 6 hours. The 53 patients received 185 infusions of CDDP.

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[3]Presented in part at the 15th Annual Meeting of the American Society of Clinical Oncology, New Orleans, LA, May 1979, and at the Symposium on Current Status and New Developments with Cisplatin, Atlanta, GA, Sept 1979.

[4]St. Jude Children's Research Hospital, Memphis, TN.

**Reprint requests to:* Charles B. Pratt, MD, St. Jude Children's Research Hospital, PO Box 318, Memphis, TN 38101.

Each patient received the initial course as an inpatient, and most patients received subsequent CDDP infusions as outpatients.

Pharmacokinetic studies of the distribution of CDDP were performed on serum samples obtained at 0, ½, 1, 4, 8, 24, and 48 hours following completion of the first 6-hour infusion for each patient. Analysis of the total platinum was made by flameless atomic absorption spectrophotometry, as previously described (6). Serial serum samples were also analyzed for free (unmetabolized, unbound) CDDP by the method of LeRoy et al (7). Serial serum concentrations of total platinum were fit to the appropriate pharmacokinetic model using the NONLIN/AUTOAN computer program.[5]

The patients were serially examined weekly or at 3-week intervals and evaluations were made of the physical examination, hemograms, hepatic and renal function studies, and chest radiographs. Audiograms were obtained at 6-week intervals.

Complete response was defined as total regression of all measurable indicators of disease activity, including bone marrow infiltration by tumor cells, for at least 4 weeks. Partial response was defined as a $> 50\%$ and $< 100\%$ regression of all measurable lesions, persisting for a period of 4 weeks. Mixed response was defined as at least partial response of some measurable indicators of disease activity, without change of additional lesions. Stable disease was defined as lack of progression of measurable indicator lesions for at least 1 month.

RESULTS

After admission of the first 25 patients, the antitumor effects and the toxic effects of CDDP were evaluated, and it was determined that these effects were similar with both treatment schedules. Accordingly, randomization was discontinued, and the additional 28 patients admitted to this study were treated at 3-week intervals to decrease patient discomfort, which was manifested primarily by nausea and vomiting associated with the weekly administration of this agent. These 28 patients received treatment without furosemide and without dextrose-containing hydration fluids.

Responses are categorized by tumor type (table 1) and by CDDP dose schedule for patients with neuroblastoma and germ cell tumors (table 2). Toxicity was evaluated in relation to the dose schedule employed (table 3).

[5]Metzler CM. NONLIN: a computer program for estimation in nonlinear situations. Technical Report No. 7297/69/7297/005. The Upjohn Co, Kalamazoo, MI, 1969.

Response to CDDP

The therapeutic benefits of CDDP were obvious in six of nine patients who received treatment for neuroblastoma (tables 1 and 2). Two patients had complete responses of 3 and 10 months, respectively, and four patients had partial responses of 8–10 weeks. The complete responses occurred in patients 3 and 16 years of age who had disease at the primary site as well as bone marrow infiltration by tumor cells; both of these patients were treated at 3-week intervals. The four patients who had partial responses had $> 50\%$ reduction of the primary area of tumor involvement; two of these four had involvement of bones as well as bone marrow. One patient was not evaluable because of early death and two failed to respond.

Partial responses were also observed in three of six patients with malignant germ cell tumors; their primary diseases were (one patient each): embryonal carcinoma of the ovary, embryonal carcinoma of the testis, and mixed malignant teratoma of the retroperitoneal area; a mixed response was achieved by a patient whose diagnosis was embryonal carcinoma of the testis.

Partial responses also were observed in one of two patients with lymphoepithelioma and in single patients with Hodgkin's disease and mesenchymoma.

In the group of 13 patients with osteosarcoma, two patients with pulmonary metastases had responses: one complete and one partial. The complete response has persisted for $> 1½$ years following four treatments at 3-week intervals. Stable disease and mixed response were recorded for two additional patients.

Stable disease was maintained by single patients with malignant carcinoid and with extraosseous chondrosarcoma.

Thirty other patients failed to achieve measurable benefit from treatment with CDDP.

Toxicity

Fifty of the 53 patients received at least two treatments with CDDP, and all patients were evaluated for toxic effects. Table 3 shows the frequency of the toxic effects observed following infusions of CDDP administered weekly or at 3-week intervals.

Fifty-one of the 53 patients had nausea or vomiting, varying in severity from minimal to severe. Vomiting generally was more severe in older patients. It was necessary to delay treatment in six of eight patients who had myelosuppression (leukopenia or thrombocytopenia). No bleeding was ob-

TABLE 1.—Response following CDDP treatment of children with malignant solid tumors

Diagnosis	No. of patients	No. of patients evaluable*	No. of responses†				
			CR	PR	MR	SD	NR
Osteosarcoma	13	13	1	1	1	1	9
Neuroblastoma	9	8	2	4	0	0	2
Rhabdomyosarcoma	9	9	0	0	1	0	8
Germ cell tumor	6	6	0	3	1	0	2
Malignant fibrous histiocytoma	3	2	0	0	0	0	2
Wilms' tumor	3	2	0	0	0	0	2
Lymphoepithelioma	2	2	0	1	0	0	1
Synovial sarcoma	2	2	0	0	0	0	2
Carcinoid	1	1	0	0	0	1	0
Chondrosarcoma	1	1	0	0	0	1	0
Mesenchymoma	1	1	0	1	0	0	0
Hemangiopericytoma	1	1	0	0	0	0	1
Hodgkin's disease	1	1	0	1	0	0	0
Ewing's sarcoma	1	1	0	0	0	0	1
	53	50	3	11	3	3	30

*Received > 1 dose of CDDP.
†CR = complete response; PR = partial response; MR = mixed response; SD = stable disease; NR = no response.

TABLE 2.—Response, by dose schedule, following CDDP in patients with neuroblastoma and germ cell tumors

Diagnosis	No. of patients responding	
	30 mg/m²/wk	90 mg/m² q3wks
Neuroblastoma	1 PR	2 CR
	2 NR	3 PR
		1 NE*
Germ cell tumor	1 PR	2 PR
	1NR	1 MR
		1 NR

*NE = not evaluable.

served in the patients who developed thrombocytopenia.

Abnormalities of hepatic function, as determined by elevated SGOT levels, were observed in six of 51 patients evaluable for this abnormality. Changes in renal function were more common and were characterized by an elevation of BUN to > 30 mg/dl. Development of renal dysfunction was related to cumulative dose of CDDP, with serious compromise of renal function observed only in patients receiving repeated infusions. Eight of 52 evaluable patients had such renal dysfunction after two to ten treatments. Only one of 52 evaluable patients had elevation of the serum creatinine to > 2 mg/dl.

Of 42 patients with sequential determinations of serum magnesium levels, 33 had abnormally low levels of this cation. Serum calcium levels were determined for 52 patients; seven had levels < 8 mg/100 ml. Six patients developed hypocalcemic, hypomagnesemic tetany, as previously reported (8). Seven additional patients complained of tinnitus, paresthesias, or leg cramps.

One patient developed an anaphylactoid reaction following initiation of the tenth CDDP infusion; laryngospasm and bronchospasm were relieved by the administration of epinephrine and dexamethasone.

Four patients developed nonfatal infections. Weight loss of 5%–19% of the pretreatment weight was recorded in 17 patients; weight loss could not be attributed to effects of CDDP in that all patients had evidence of advancing disease.

Twenty-two patients were monitored with serial audiograms; minimal to severe loss of high-frequency tone appreciation was detected in nine patients, and three had functional hearing loss.

One patient developed severe abdominal cramping associated with diarrhea within 12 hours of the CDDP infusion. These symptoms were relieved with the administration of narcotics. An additional patient developed perianal pigmentation 1 week following his first treatment with CDDP.

Pharmacokinetic Studies

CDDP serum concentrations were assayed following 20 infusions in 14 patients receiving doses of 90 mg/m² and following nine infusions in nine patients receiving doses of 30 mg/m². The data were fitted to a biexponential equation following 11 courses in nine patients. Figure 1 shows the mean serum concentrations achieved in the 14 patients during the 48 hours following the end of the infusion of 90 mg/m² of CDDP. The SD values are indicated by the bars on the vertical axes. Comparison of the values

TABLE 3.—Toxicity following treatment with CDDP*

Toxic effect	Schedule	
	30 mg/m²/wk	90 mg/m² q3wks
Nausea, vomiting	9/10	42/43
Hematopoietic depression		
Hgb < 8 g/100 ml	3/9	7/38
Wbc count < 2000/mm³	2/10	5/43
Platelet count < 100,000/mm³	3/10	4/41
Hepatic function abnormalities	2/10	4/41
Renal function abnormalities		
BUN > 30 mg/dl	1/10	7/43
Creatinine > 2 mg/dl	0/10	1/42
Mg^{2+} < 1.4 mEq/liter	6/6	27/36
Ca^{2+} < 8 mg/100 ml	4/10	3/42
Neuromuscular		
Tetany	3/10	3/43
Paresthesia	4/10	5/43
Allergy	0/10	1/43
Infection	1/10	3/43
Weight loss > 5% pretreatment weight	4/10	13/43
Abnormal audiogram	3/4	6/18

*Values = No. of patients who had toxic effects/No. of patients evaluable.

for the patients treated with doses of 90 and 30 mg/m² is shown in figure 2.

Mean (± SEM) half-life values of total platinum for the patients studied were 24.9 (± 6.2) minutes for the α (initial) phase and 44.4 (± 8.2) hours for the β (terminal) phase. Mean (± SEM) half-life of free unbound platinum was determined to be 1.3 (± 0.4) hours.

No acute toxic effects could be related to the serum concentrations of CDDP.

DISCUSSION

The efficacy of CDDP was most notable following treatment of children with neuroblastoma and malignant germ cell tumors, although complete or partial responses also occurred in patients with osteosarcoma, Hodgkin's disease, mesenchymoma, and lymphoepithelioma. Responses by children with neuroblastoma, malignant germ cell tumors, and osteosarcoma have previously been reported by other investigators (9–15).

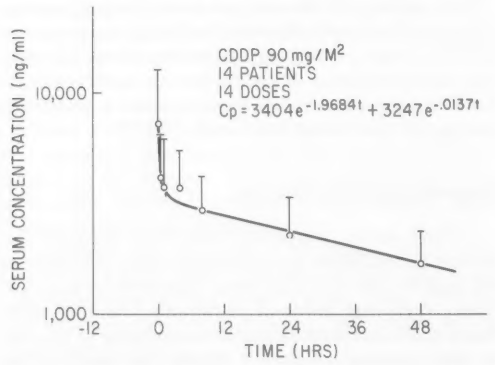

FIGURE 1.—Serum disposition curve for semilogarithmic plot of total platinum over 48 hours for 14 patients who received CDDP in a dose of 90 mg/m².

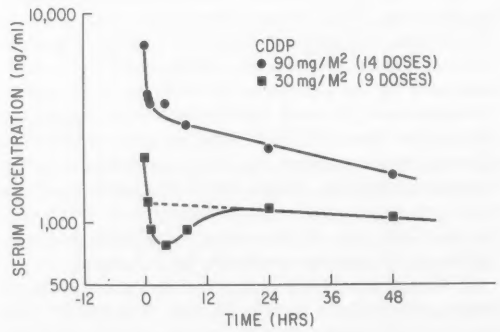

FIGURE 2.—Comparison of total platinum serum disposition for children receiving CDDP at doses of 90 and 30 mg/m².

CDDP administered by weekly infusion or as a single dose every 3 weeks was tolerated by children with advanced solid tumors previously treated with other therapies. Except for the single incidence of anaphylaxis and the six episodes of hypomagnesemic, hypocalcemic tetany, toxic effects were neither severe nor dose-limiting (16–19). The hypomagnesemic, hypocalcemic tetany has not been observed since we have routinely administered daily doses of magnesium gluconate (3 g/m²) to all patients receiving CDDP.

Although two of the nine responses in patients with neuroblastoma and malignant germ cell tumor were achieved in patients treated with the weekly schedule of CDDP, the impact of weekly treatment on the nutritional status of these patients, including poor oral intake with weekly vomiting, was sufficient to alter the treatment schedule. After the change of schedule, weight loss was not as significant a problem for the 28 patients who received doses of 90 mg/m² of CDDP in the nonrandomized continuation of the study.

Our pharmacokinetic studies have indicated an initial half-life of about 30 minutes with a second-phase half-life of approximately 44 hours for total platinum. Assessment of the distribution of CDDP in these patients demonstrates that the disposition of total platinum is best described as a multicompartmental model. In most patients the distribution into the tissue compartment occurred at a rate > 6 times the rate of exit from tissues. The resulting β-half-life (44 hours) is similar to the value reported for adults (20). The half-life of free unchanged CDDP was determined to be approximately 1.3 hours. Serum half-life values for the two dose schedules were not different, although peak levels were proportionally higher for patients receiving the higher dose at 3-week intervals.

Correlation of pharmacokinetic parameters with age was attempted using β-half-life and α-half-life and K_{12}, K_{21}, and K_e rate constants from the 11 serum curves which were fitted to a two-compartmental model. K_e was significantly correlated ($P < 0.05$) with age, as a larger value for K_e (more rapid elimination) was seen in older patients. However, only one patient was < 2 years of age at the time of study, and this sample size ($N = 11$) is much too small to yield conclusive results, making the significance of this observation uncertain.

CDDP has been shown to be active in ovarian and testicular germ cell tumors of adults and children (3,12,21). Failure to observe responses in large numbers of patients with soft tissue sarcomas and osteosarcoma was consistent with the results of previous trials (11,14,15,22). Based on the quality of responses of the patients with neuroblastoma and malignant germ cell tumors, it can be recommended that CDDP be used more extensively in children receiving combination chemotherapy for these diseases.

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Phase II Study of Hexamethylmelamine in Refractory Hodgkin's Disease, Other Lymphomas, and Chronic Lymphocytic Leukemia[1,2,3]

G. A. Omura,* G. O. Broun, J. Papps, and R. Birch[2]

Writing Committee for the Southeastern Cancer Study Group

SUMMARY

A long-term daily dose of hexamethylmelamine was tested in patients with Hodgkin's disease, nodular and diffuse lymphomas, and chronic lymphocytic leukemia who had failed conventional treatment. There were no responses among 20 patients with chronic lymphocytic leukemia, 12 with nodular lymphomas, and 11 with diffuse well-differentiated lymphocytic lymphomas. However, five of 24 patients with Hodgkin's disease (21%) responded and seven of 36 (19%) with diffuse lymphomas other than diffuse well-differentiated lymphocytic lymphomas responded. This new agent is active in selected histologic types of lymphoma.

[Cancer Treat Rep 65:1027–1029, 1981]

Hexamethylmelamine (HXM) is a congener of the alkylating agent triethylenemelamine and is of interest clinically because it produces tumor regression in some cancers refractory to mustard-type agents. Only small groups of patients with lymphoma have been tested in the various trials reported to date. Wampler et al (1) described one complete remission (CR) in four patients with Hodgkin's disease (HD). Bergevin et al (2) reported a partial response (PR) in one patient with nodular lymphoma, while two with reticulum cell sarcoma had disease progression. Borden et al (3), recapitulating earlier experience of the Central Oncology Group, reported one CR and six PRs in seven patients with HD and one CR plus four PRs in seven patients with non-Hodgkin's lymphoma (3). Further evaluation was indicated to better define response rates in lymphomas; we evaluated chronic lymphocytic leukemia (CLL) as well.

MATERIALS AND METHODS

Adults with biopsy-proven HD, non-Hodgkin's lymphoma, or CLL in relapse after failure of stand-

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[2]This study was conducted by the Southeastern Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Alabama, Birmingham (*G. A. Omura, R. Birch, J. Barton, R. Burson, J. Carpenter, M. Conrad, J. Durant, R. Gams, D. Lineberry,* and *E. Knight,* CA-03013); St. Louis University, MO (*G. O. Broun,* CA-17214); Veterans Administration Hospital, Brooklyn, NY (*J. Papps, D. Babott, I. Esseesse,* and *L. Meyer*); Duke University, Durham, NC (*H. Silberman,* CA-03177); Veterans Administration Hospital, Durham, NC (*H. Cohen,* CA-05634); Emory University, Atlanta, GA (*L. Cooper, T. Heffner, H. Kann,* and *D. Nixon,* CA-03227 and CA-11263); University of Miami, FL (*H. Lessner* and *M. Troner,* CA-05641); Temple University, Philadelphia, PA (*F. LaLuna, R. Okpara,* and *R. Smalley,* CA-07961); Washington University, St. Louis, MO (*V. Loeb, Jr,* and *C. Presant,* CA-03376); University of Puerto Rico, San Juan (*A. Grillo-Lopez, F. Robert,* and *E. Velez-Garcia,* CA-12223); Rush Medical Center, Chicago, IL (*S. Gregory, W. Knospe, J. Weems,* and *M. Westerman,* CA-12640); Veterans Administration Hospital, New Orleans, LA (*K. Tornyos,* CA-13249); Case Western Reserve University, Cleveland, OH (*R. Kellermeyer* and *A. Rassiga,* CA-15584); University of Louisville, KY (*S. Lowenbraun,* CA-16389); University of Tennessee, Memphis (*L. Burkett* and *C. Neely,* CA-17027); University of Kentucky, Lexington (*P. DeSimone* and *J. Hutton,* CA-20255); University of Cincinnati, OH (*M. Denton* and *O. Martelo,* CA-28138); and University of South Florida, Tampa (*G. Lyman*).

[3]Hexamethylmelamine was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* George A. Omura, MD, Department of Medicine, Division of Hematology and Oncology, The University of Alabama in Birmingham, 638 Zeigler Bldg, University Station, Birmingham, AL 35294.

TABLE 1.—Responses of 103 evaluable patients to HXM

Diagnosis	Evaluable	Adequate trial (≥ 6 wks)	CR	PR	No change	Disease progression	Died
HD	24*	17	1	4	6	9	3
Diffuse lymphomas	47	24	0	7	14	24	2
Nodular lymphomas	12	8	0	0	6	4	2
CLL	20	13	0	0	6	12	2

*Values = No. of patients.

TABLE 2.—Patient characteristics

Diagnosis	No. of evaluable patients	Median age in yrs (range)	Sex (% male)
HD	24	30 (23–74)	71
Diffuse lymphomas	47	55 (22–78)	74
Nodular lymphomas	12	56 (37–77)	58
CLL	20	59 (46–80)	85

ard therapy were eligible if they had at least one measurable lesion (lymphocytosis > 15,000/mm³ in patients with CLL), had recovered from the toxic effects of prior treatment, had a Karnofsky performance status of at least 40% and an estimated survival of at least 6 weeks, had no major impairment of renal or hepatic function, and had given written informed consent for the study. Central pathology review was not done, but most of the patients had previously been in other Southeastern Group studies where this was required.

HXM (280 mg/m²) was given orally in four divided daily doses for at least 6 weeks or until disease progression or severe toxic effects occurred. The total dose for each 3-week period was calculated to the nearest 100-mg capsule. Cbcs were performed weekly, tumor measurements were made every 3 weeks, and relevant radiographs were obtained every 6 weeks. If the granulocyte count decreased to 1500–2000/mm³ or if the platelet count decreased to 75,000–100,000/mm³, the dose was halved. If the granulocyte count decreased to < 1500/mm³ or if the platelet count decreased to < 75,000/mm³, treatment was interrupted and resumed at one-half dose when the blood counts exceeded those levels. A CR was defined as the disappearance of all measurable disease; PR was defined as at least a 50% reduction in the sum of the products of the perpendicular diameters of measurable lesions. In patients with CLL, a PR was defined as a ≥ 50% reduction in nodal disease and splenomegaly and a wbc count < 50% of the initial level.

RESULTS

Of 138 patients entered in the study, 12 had incomplete data, six were ineligible, five had major protocol violations, five had intercurrent diseases preventing treatment, three refused treatment, and four were lost to followup. The responses of the 103 evaluable patients are shown in table 1. The age range and sex distribution are shown in table 2. The response durations for HD were 15, 15+, 21, 22, and 82 weeks, while those for diffuse lymphomas were 6, 10, 16, 17, 37, 40, and 41 weeks. Among the patients with diffuse lymphomas, there were no responses in 11 patients with well-differentiated lymphocytic, three PRs in 17 with poorly differentiated lymphocytic, one PR in nine with diffuse histiocytic, two PRs in seven with diffuse mixed, and one PR in the one patient with diffuse undifferentiated lymphoma. Two patients with diffuse lymphomas whose histology was not more detailed did not respond.

Thus, if the last two patients and those with well-differentiated histology are excluded, seven of 34 patients (21%) with diffuse lymphomas responded. Most of the patients (62) continued treatment at least for the duration of our arbitrarily defined adequate trial (6 weeks). One additional HD patient responded for 1 week but developed severe neurotoxicity; when treatment was stopped, the toxic effects cleared and the disease progressed rapidly. Three patients with nodular lymphomas showed

TABLE 3.—Toxicity

Toxic effect*	HD + lymphoma (83 patients)	CLL (20 patients)
Granulocytopenia		
1+	15	2
2+	6	
Thrombocytopenia		
1+	14	6
2+	14	3
Nausea and vomiting		
1+	19	6
2+	28	4
3+	3	2

**Granulocytopenia*: 1+ = decrease in count of segmented and band cells from normal to 750–1500/mm^3; 2+ = count < 750/mm^3. *Thrombocytopenia*: 1+ = decrease in count from normal to 50,000–100,000/mm^3; 2+ = count < 50,000/mm^3. *Nausea and vomiting*: 1+ = no treatment required; 2+ = controlled by antiemetics; and 3+ = not controlled.

minor responses but there were no CRs or PRs. No patient with CLL responded.

Hematologic and gastrointestinal toxicity are shown in table 3. One CLL patient developed persistent rbc aplasia after 4 weeks of treatment. Ten patients developed neurologic effects that were attributed to HXM, including tremor and hallucinations, paresthesias, paranoid ideation, disorientation, headache and confusion, depression, and lethargy in individual patients and ataxia in three others. These effects subsided when the drug was stopped. Pyridoxine was not used prophylactically. One CLL patient had a marked elevation of serum alkaline phosphatase without other explanation.

DISCUSSION

Our experience with HXM in the present trial confirmed that this drug has activity in HD and in certain non-Hodgkin's lymphomas, despite the fact that previously heavily treated patients were included. The importance of identifying specific lymphoma histologies in phase II trials was underscored by our finding that no patients with nodular or well-differentiated lymphomas and none with CLL responded. Since responses in CLL may occur slowly, it might be questioned whether 6 weeks was an adequate trial period, but most of these patients did have disease progression during therapy, suggesting that the trial was indeed adequate. The long-term daily administration of HXM was troublesome with respect to cumulative nausea and vomiting as well as hematologic and occasional neurologic toxicity. An intermittent schedule, such as has been successfully used in ovarian cancer (4), should be considered for further evaluation in combination regimens for HD and lymphomas with poor prognosis.

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Phase I and Pharmacokinetic Studies of DON[1,2]

John S. Kovach,* Robert T. Eagan, Garth Powis, Joseph Rubin, Edward T. Creagan, and Charles G. Moertel[3]

SUMMARY

DON, a glutamine antagonist, was administered iv to 26 patients with advanced cancer either once every 3 wks or daily for 3 days every 3 wks to determine toxicity and to look for evidence of therapeutic effect. Total doses ranged from 150 to 600 mg/m². The single-day schedule produced intolerable nausea and vomiting and no evidence of cytotoxicity at 450–550 mg/m² given over 10 mins or over 4 hrs. On the 3-day schedule, patients had tolerable gastrointestinal toxic effects at total doses up to 480 mg/m² given in three equally divided doses by 10-min infusion. This dose also produced cytotoxic activity manifested as transient mild leukopenia and, rarely, thrombocytopenia. No objective responses were seen. Analysis of the plasma elimination of DON demonstrated dose-dependent pharmacokinetic behavior. The parent compound was not detectable in the urine of any patient, indicating extensive metabolism of the drug.

[Cancer Treat Rep 65:1031–1036, 1981]

DON inhibits the activity of several L-glutamine amidotransferases concerned with purine and pyrimidine biosynthesis and is a potent inhibitor of L-asparagine synthetase (1). Extensive studies of the mechanism of action and in vitro metabolism of DON have been conducted by the Laboratory of Toxicology, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), and the Southern Research Institute (Birmingham, AL) (2–4). The development of DON as a clinical anticancer agent has been recently reviewed by Burchenal (5) and Catane et al (6). DON is active against L1210 murine leukemia (5) and against the human tumor xenografts LX-1 (lung carcinoma), CX-2 (colon carcinoma), and MX-1 (breast carcinoma) in nude mice (6). In phase I trials conducted over two decades ago, DON was administered primarily by the oral route, although some patients received the drug or iv (5). Usually, the drug was given daily at doses of 0.1–0.3 mg/kg/day. This regimen produced predominantly mucositis and to lesser extents diarrhea, nausea, vomiting, and bone marrow suppression. In a phase II study Magill et al (7) noted responses in two of 14 patients with breast cancer, in two of nine with lung cancer, in one of six with colon cancer, in one of five with uterine cancer, and in one of four with lymphoma. Three patients with choriocarcinoma had complete responses, with two apparently being cured (5). Sullivan et al (8) reported that 63 of 94 children with acute leukemia achieved complete remission following treatment with a combination of DON and 6-mercaptopurine. There was only a slight advantage of the combination over 6-mercaptopurine alone and the combination produced more toxic effects, particularly mucositis.

Burchenal (5) reported that in the L1210 and P388 murine leukemia models, much more drug could be administered on a 2- or 4-day schedule; these schedules produced better therapeutic results than a daily schedule. Because of its activity against several human tumor xenografts and because it had not been studied on any intermittent schedules, DON was studied in phase I trials at several institutions. We initially studied DON administered by 10-min

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[3]Department of Oncology, Mayo Clinic, Rochester, MN.

Reprint requests to: John S. Kovach, MD, Department of Oncology, Mayo Clinic, Rochester, MN 55901.

and by 4-hr infusions once every 3 wks. Because of intolerable nausea and vomiting on this schedule, we then studied DON given by 10-min infusion daily for 3 consecutive days. Plasma elimination of DON was studied at multiple dose levels in the single-day schedule of administration.

PATIENTS AND METHODS

Patients

All patients had histologic or cytologic confirmation of unresectable cancer for which no other more conventional means of therapy offered reasonable hope of cure or of significant palliation. All patients were required to be ambulatory and to be maintaining a reasonable state of nutrition (caloric intake > 1200 calories/day). Patients were excluded for the following reasons: wbc count < 4100/mm^3, platelet count < 130,000/mm^3, hemoglobin < 10 g/dl, any elevation of direct-reacting serum bilirubin, serum creatinine ≥ 1.5 mg/dl, performance status of 4 (Eastern Cooperative Oncology Group scale), major surgery within 30 days, radiation to > 15% of bone marrow within 30 days, previous significant radiation to > 30% of bone marrow (the equivalent of 1500 rads in 13 fractions), therapy with cytotoxic agents within 30 days, uncontrolled infection, frequent vomiting or anorexia, or any condition which precluded obtaining informed consent. Within 3 days of treatment Day 1 all patients had a complete physical examination including measurement of tumors, whenever possible, and wbc count, hemoglobin, platelet count, SMA-12, chest roentgenogram, ECG, serum amylase, and urinalysis. All patients were seen by a physician prior to each dose of DON and 24 hrs after the last dose of DON on each schedule. Patients in the 3-day schedule had a wbc count, hemoglobin, platelet count, and chemistry group on Day 4. During the interval between 3-wk cycles of drug, wbc counts, hemoglobin, and platelet counts were obtained twice weekly and serum creatinine and SGOT were obtained once weekly. On at least Day 1 of treatment at any new dose of DON, all patients had pulse, temperature, and blood pressure determined prior to each dose and standing and recumbent pulse and blood pressure determinations 15 mins following drug administration and then every 30 mins for 4 hrs.

In each regimen patients were entered in groups of three at each dose level. New patients were entered at a higher dose level only after the three patients treated at the previous level had been observed for a minimum of 3 wks, counting from the last day of treatment. Dose was escalated in increments of 50% until the first evidence of toxicity was noted in any of the three patients treated at the previous level. When toxicity was seen, the rate of dose escalation was reduced, depending on the nature and duration of the toxicity, but in no case exceeded 25% of the previous level. Re-treatment of any patient was based on therapeutic response and on the type, severity, duration, and reversibility of any toxic reaction. Patients who showed improvement or stability were continued in the study, the dose of subsequent treatments being determined by the toxicity noted following previous treatment. Any patient showing objective progression of disease or clinical deterioration was not re-treated with DON. No other cytostatic, cytotoxic, or immunostimulatory agent was administered while the patient was in the study. All other medications such as analgesics and antiemetics were permitted, as clinically indicated.

Dose and Schedule of Treatment

In the single-day schedule, DON was administered by iv infusion over 10 mins at doses of 300, 450, and 550 mg/m^2 and by infusion over 4 hrs at doses of 300 and 450 mg/m^2. On the daily × 3-day schedule DON was studied at total doses (> 3 days) of 150, 225, 330, 480, and 600 mg/m^2. In both schedules re-treatment was planned at 3 wks from Day 1 of drug administration.

Pharmacologic Studies

DON was measured by a high-performance liquid chromatographic method described previously (9), which is based on the reverse-phase separation, on a C_8-bonded column, of the dansyl derivative of DON [dansyl chloride; 5-(dimethylamino)-1-naphthalenesulfonyl chloride] from other dansyl derivatives in the plasma and urine. Blood samples were withdrawn into heparin-containing tubes. Plasma was separated by low-speed centrifugation and stored at –70°C for no more than 24 hrs before assay. Patients receiving DON by 10-min infusion had a 4-ml venous blood sample taken immediately prior to treatment and then at 1, 5, 10, 15, 30, 60, 120, 180, 240, and 360 mins and 24 hrs. Patients receiving the drug by 4-hr infusion had a blood sample taken immediately prior to treatment, at 60, 90, 120, 180, and 240 mins of infusion, and then at 10, 20, 30, 45, 60, 90, 120, and 180 mins and 20 hrs after the infusion was stopped. Spontaneously voided samples of urine were collected separately up to 6 hrs after drug administration and then as a single sample for 24 hrs.

Pharmacokinetic analysis of the data was conducted using the SAS NLIN (10) computer program. The biexponential decline in the plasma concentration of DON was fitted by a nonlinear least-squares regression analysis to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$. C is the plasma concentration of DON at time t after administration. A and B are the intercepts at t = 0, and α and β are the fast and slow disposition rate constants. A weighting factor of C^{-1} was used. Allowance was made for an infusion time of 10 mins or 4 hrs.

Drug Supply and Preparation

The drug was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, as a lyophilized powder in vials containing 100 mg of DON. It was dissolved in 50 ml of 0.9% saline for 10-min infusions and in 250 ml of 0.9% saline for 4-hr infusions.

RESULTS

Gastrointestinal Toxicity

Seven patients received DON by 10-min infusion in a single-day schedule to be repeated every 3 wks. As shown in table 1, three patients received their initial course at 300 mg/m², three patients at 450 mg/m², and one patient at 550 mg/m². Mild to moderate vomiting was seen in two of three patients treated at the lowest dose. Vomiting became more severe at a dose of 450 mg/m² and was intolerable at a dose of 550 mg/m². One patient initially treated at a dose of 300 mg/m² had two subsequent treatments at 450 mg/m², with moderate nausea and vomiting. One patient initially treated at a dose of 450 mg/m² refused to consider any further chemotherapy. The single patient treated at a dose of 550 mg/m² had severe and persistent nausea and vomiting requiring hospitalization for hydration. At this point we abandoned the single-day 10-min infusion regimen and studied DON given by 4-hr infusion once every 3 wks. Two patients tolerated a dose of 300 mg/m² by 4-hr infusion with only mild nausea and vomiting, but two new patients treated at a dose of 450 mg/m² by 4-hr infusion experienced moderate to severe nausea and vomiting following two successive courses at this dose and refused to consider any further chemotherapy. Because of lack of significant cytotoxic effects (leukopenia, thrombocytopenia, or significant stomatitis) and marked gastrointestinal toxicity in the single-day schedule by either 10-min or 4-hr infusion, we initiated a study of DON given by a 10-min infusion daily for 3 consecutive days.

A total of 16 patients (ten women and six men) with an Eastern Cooperative Oncology Group status of 2 or better were entered in the study. The starting dose was 150 mg/m² over 3 days, the dose being lower than that initially used in the single-day schedule since marked cumulative toxicity had been reported for DON in multiple-day schedules in mice (4). As shown in table 2, significant vomiting was first encountered in two of four patients initially treated at a dose of 330 mg/m². Re-treatment of one patient at a total dose of 150 mg/m², three at 225 mg/m², and one at 330 mg/m² produced in each instance no greater gastrointestinal toxicity than seen with the initial course.

Up to this point patients in the 3-day schedule did not routinely receive parenteral antiemetics prior to their first drug dose, although all patients experiencing nausea and vomiting following their initial treatment received parenteral antiemetics prior to subsequent days of treatment. Total dose was escalated to 480 mg/m² and all patients received 10 mg of prochlorperazine im approximately 30 mins prior to the initial treatment. Of three patients treated at this dose, two had moderate but tolerable nausea and vomiting and one had no symptoms. The patient experiencing no gastrointestinal symptoms had a myocardial infarction the day following completion of therapy. She had an uneventful recovery and 6 wks later reported marked diminution in pain from a destructive lesion in the lumbar spine due

TABLE 1.—Gastrointestinal toxicity after DON by 10-min infusion on a single day

Total dose (mg/m²)	No. of first courses	Vomiting (0–3+)*
300	3	0,1+,2+
450	3	2+,2+,3+
550	1	3+

* Grading of vomiting: 0 = none; 1+ = 1–3 times; 2+ = 4–10 times; 3+ = > 10 times (per 24 hrs).

TABLE 2.—Gastrointestinal toxicity after DON by 10-min infusion daily for 3 days

Total dose (mg/m²)	No. of first courses	Vomiting (0–3+)*
150	3	0,0,1+
225	3	0,1+,2+
330	4	0,1+,3+,3+
480	3†	0,2+,2+
600	3†	3+,3+,3+

* Grading of vomiting: *see* table 1 for definitions.
† Parenteral prochlorperazine before each dose.

to a fibrosarcoma. Re-treatment with DON at a dose of 480 mg/m² produced moderate to severe nausea and vomiting and no other toxic effect.

Total dose was escalated to 600 mg/m² in three new patients. All three patients experienced nausea and vomiting which tended to become increasingly severe with each day of therapy, despite parenteral antiemetics. Symptoms persisted for approximately 10 days and all three patients refused further therapy with the drug.

The maximum tolerable total dose appeared to be 480 mg/m² (160 mg/m² by 10-min infusion daily for 3 days). Since in the single-day schedule we had found that the dose of 300 mg/m² was well-tolerated as a single 4-hr infusion, we planned to study three additional patients receiving DON at a dose of 160 mg/m² by 4-hr infusion on 3 consecutive days. The first patient studied had increasingly severe nausea and vomiting over the treatment period and for 4 more days. He had an acute upper gastrointestinal hemorrhage of unknown etiology 3 days after completion of treatment, which ceased with conservative management. We did not study the 4-hr infusion regimen further.

Hematologic Toxicity

As shown in table 3, mild transient leukopenia occurred at all dose levels of DON administered by 10-min infusions in the 3-day schedule. One patient with carcinoma of the rectum previously treated with pelvic irradiation and 5-FU had no hematologic toxic effects after an initial course at 330 mg/m². Twenty-one days after a second course at 412.5 mg/m², she had a leukopenia nadir of 1900 cells/mm³. Leukopenia was seen consistently only at a total dose of 480 mg/m². Mild decreases in platelet counts were seen at all dose levels up to 480 mg/m², but in each instance platelet counts were not below the lower limits of normal. The one patient who received a total dose of 480 mg/m² for 3 consecutive 4-hr infusions and had an upper gastrointestinal hemorrhage had a thrombocytopenia nadir of 63,000 platelets/mm³ on Day 9, with recovery on Day 12. He did not have leukopenia, although the wbc count decreased from 27,100 to 10,400/mm³ on Day 7, returning to 17,000/mm³ on Day 12.

Incomplete hematologic data were obtained for the three patients treated at a total dose of 600 mg/m² over 3 days. One patient refused further tests and refused to return for further treatment. A second patient had persistent nausea and vomiting and did not report for followup laboratory studies until 13 days after initiation of therapy, at which time all counts were normal. A third patient who had had extensive prior chemotherapy had a platelet count of 11,000/mm³ and a wbc count of 8600/mm³ 10 days after the first day of treatment. Thrombocytopenia was not complicated by hemorrhage. Seventeen days after treatment the platelet count was 105,000/mm³ and the wbc count was 9700/mm³; at 21 days the counts had completely recovered.

Other Toxic Effects

One patient treated twice with DON at a dose of 225 mg/m² over 3 days reported a marked decrease in recent memory for 1 wk following both courses. This unusual symptom was not elicited until 3 wks after the second course at which time she was asymptomatic. Subsequent treatment with other chemotherapeutic agents did not produce memory loss. One patient treated at a dose of 600 mg/m² over 3 days had marked blurring of vision for 48 hrs after completing therapy. She had no other neurologic symptoms. Vision returned to normal. She was not re-treated with DON because of progressive disease. DON did not appear to cause alteration in renal or hepatic function in any patient.

Therapeutic Responses

No objective responses to DON were seen in any regimen. In the 3-day schedule, a woman with lo-

TABLE 3.—Hematologic toxicity after DON by 10-min infusion daily for 3 days

Total dose (mg/m²)	No. of evaluable initial courses	No. of courses with wbcs < 4.1 × 10³/mm³ (wbc count nadir, × 10³ cells/mm³)	No. of courses with platelets < 130 × 10³/mm³ (platelet count nadir, × 10³ cells/mm³)
150	3	2 (3.1, 3.6)	None
225	3	None	None
333	3	1 (3.7)	None
480	3	3 (3.1, 3.5, 3.9)	1 (125)
480 (4-hr infusion)	1	None	1 (63)

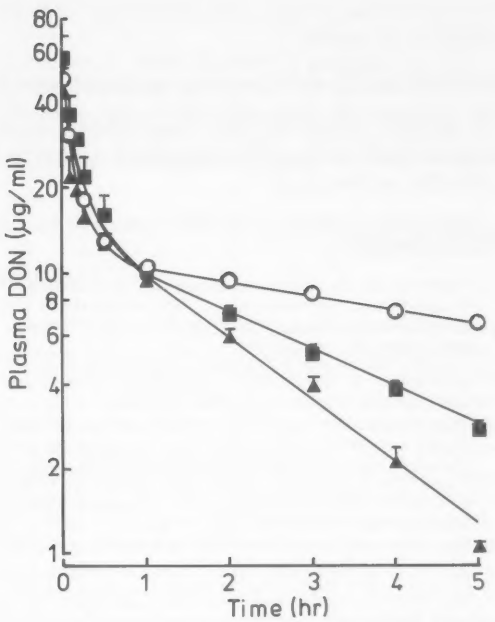

FIGURE 1.—Concentration of DON in plasma after rapid injection in 3 patients at 300 mg/m² (▲), 3 patients at 450 mg/m² (■), and 1 patient at 550 mg/m² (○). (I) = SEM.

cally recurrent fibrosarcoma eroding the lumbar spine had marked decrease in chronic pain after a dose of 160 mg/m² daily for 3 days. She was re-treated at 6 wks (delayed because of myocardial infarction) at the same dose. Progressive disease was documented at 9 wks. A 44-year-old man with adenocarcinoma of unknown origin initially treated at a dose of 330 mg/m² over 3 days remained stable for 10 wks.

Pharmacologic Studies

Plasma concentrations of DON in patients receiving the drug as a 10-min infusion are shown in figure 1. The data were approximated best by a two-compartment open model with elimination from the central compartment. The pharmacokinetic parameters are shown in table 4. DON appears to exhibit dose-dependent pharmacokinetics with a prolonged distributive half-life and a decreasing clearance as the dose is increased. The small number of patients studied at each dose level does not allow a definite conclusion to be reached. No DON was detected in the plasma 24 hrs after administration of the drug and no unchanged DON was detected in either the 6- or the 24-hr urine samples at any dose level. Plasma concentrations of DON in three patients receiving a 4-hr iv infusion at a dose of 300 mg/m² are shown in figure 2. The limited data obtained do not allow a meaningful estimate of the individual pharmacokinetic parameters. At this dose, the peak plasma concentration was between 5 and 10 µg/ml by the end of the 4-hr infusion. The post-distributive plasma half-life was approximately 65 mins. No DON was detected in plasma 20 hrs after the infusion was stopped, and no unchanged DON was found in the urine.

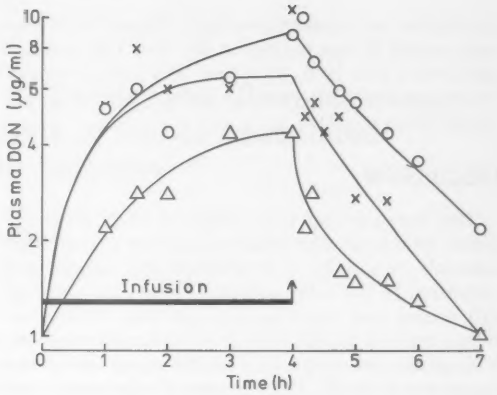

FIGURE 2.—Concentration of DON in plasma of 3 patients during and after iv administration of 300 mg/m² over 4 hrs.

A limited number of blood samples were obtained during drug infusion on Days 1 and 3 from the patient receiving DON at a dose of 160 mg/m² by 4-

TABLE 4.—Pharmacokinetic parameters of DON administered by rapid iv injection*

Dose (mg/m²)	No. of patients	α-half-life* (mins)	β-half-life* (mins)	Vss (ml/kg)	Cl (ml/min/kg)
300	3	1.6	76.2	449	3.39
450	3	5.2	105.9	766	3.56
550	1	5.9	320.5	1008	1.98

* α-half-life = distributive half-life; β-half-life = postdistributive half-life; Vss = steady state volume of distribution; Cl = whole-body clearance.

hr infusion on 3 consecutive days. Plasma DON concentrations in this patient at 60, 120, 180, and 240 mins were 9.0, 10.0, 10.4, and 10.6 μg/ml, respectively, on Day 1, and 9.0, 10.3, 9.6, and 11.0 μg/ml, respectively, on Day 3.

DISCUSSION

The dose-limiting toxic effect of DON given by either 10-min or 4-hr infusion in either a single-day schedule or a daily × 3 schedule was nausea and vomiting. In the 3-day schedule (total dose, 480 mg/m^2) nausea and vomiting was tolerable. Three patients treated at this dose level had mild transient leukopenia. At comparable total doses given by the single-day schedule, DON produced only nausea and vomiting without any evidence of cytotoxic effects. Administration of drug by 4-hr infusion in the single-day or in the 3-day schedule did not decrease the severity of gastrointestinal symptoms.

Our pharmacologic studies using a high-pressure liquid chromatographic assay specific for intact DON are in good agreement with the data obtained by Magill et al (7) using a microbiologic assay, which were reported over two decades ago. They measured plasma elimination of DON after iv doses considerably lower than those used in this study. They noted biphasic patterns of elimination and peak plasma concentrations of antimicrobial activity after 0.2–0.3 mg/kg and 0.6–1.25 mg/kg of 0.25–0.5 and 1.5–6.8 μg/ml, respectively. They also detected little, if any, DON in 24-hr urine samples. Our data on plasma elimination after doses of 300, 400, and 550 mg/m^2 are compatible with dose-dependent pharmacokinetic behavior. The mechanism responsible for this phenomenon and its relationship to increasingly severe gastrointestinal toxicity with increasing dose are unknown. Additional studies on the plasma elimination of DON for several days during and after multiple-day schedules of administration and studies of the in vivo metabolism of DON would be of interest.

We are planning to conduct phase II studies of DON at a dose of 160 mg/m^2 by 10-min infusion daily for 3 consecutive days. We believe that some patients may tolerate this dose with minimal toxic reaction, and we recommend increasing the dose by 10%–20% in this group.

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Assessment of In Vivo Effectiveness of Tumoricidal Chemotherapy and Radiation Therapy by Serial Analysis of Tumor-Associated Urinary Antigen Titers in Patients With Sarcoma[1,2]

James F. Huth,* Rishab K. Gupta, and Donald L. Morton[3]

SUMMARY

Serial measurements of tumor-associated antigens in the urine of patients with sarcoma who received preoperative intra-arterial doxorubicin and radiation therapy were assayed by microcomplement fixation. Changes in urinary antigen titer as a result of therapy were compared to pretreatment samples and were correlated with clinicopathologic evidence of in situ tumor cell destruction. Of the 53 patients with sarcoma studied, 44 had clinicopathologic evidence of tumor destruction induced by the preoperative therapy, and all 44 had a fourfold or greater rise in the level of urinary antigens during the treatment period. The other nine patients had no evidence of tumor destruction and antigen titers remained unchanged. Results suggested that tumoricidal therapy released tumor-associated antigens which could be detected in urine by microcomplement fixation. This phenomenon may be useful for measuring the in vivo effectiveness of tumoricidal therapy on nonaccessible tumors.

[Cancer Treat Rep 65:1037–1042, 1981]

Several investigators have detected novel proteins (1), glycoproteins (2), and tumor-associated antigens (3–5) in the urine of cancer patients by a variety of biochemical and immunologic techniques. In a preliminary study using the complement fixation assay, we demonstrated tumor-associated antigens in the urine of sarcoma patients with large tumor burdens. This antigen activity disappeared after surgical ablation of tumor (6). This finding suggested that tumor-associated antigens were shed from neoplastic cells during the process of cell turnover, gained access to the bloodstream, and were subsequently excreted into the urine.

Our experience in the treatment of patients with soft tissue and skeletal sarcomas has shown that the intra-arterial infusion of doxorubicin followed by radiation therapy induced in situ tumor cell destruction (7) and probably released tumor-associated antigens that can be detected in the urine.

This investigation was designed to measure sequential changes in the level of tumor-associated antigens in the urine during preoperative infusion of doxorubicin and subsequent radiation therapy in patients with sarcoma. Changes in antigen titer were correlated with the tumor cell destruction determined by histologic inspection of the resected tumor specimen.

MATERIALS AND METHODS

Patients and Treatment

Fifty-three consecutively treated patients (31 males and 22 females) with bone and soft tissue

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[3]Division of Oncology, Department of Surgery, University of California School of Medicine, Los Angeles; and the Surgical Service, Veterans Administration Medical Center, Sepulveda, CA.

Reprint requests to: James F. Huth, MD, Division of Oncology, Department of Surgery, 54-140 CHS, University of California School of Medicine, Los Angeles, CA 90024.

sarcomas were included in this study. The histologic classifications of their tumors are listed in table 1. Preoperative treatment with doxorubicin (30 mg/day × 3 days) infused into the main artery feeding the tumor and with radiation therapy (350 rads/day × 10 days) preceded radical resection of tumor (fig 1). Representative areas of each resected tumor specimen were examined microscopically to assess the effect of preoperative treatment. Pretreatment and posttreatment tissue slides were reviewed by one pathologist without prior knowledge of diagnosis or treatment received. Visual estimates of the percent of tumor necrosis per slide area were made and averaged for the total number of slides. Tumor necrosis was defined as total absence of identifiable nuclei. The degree of necrosis was then correlated to the change in antigen titer.

Urine Samples

Twenty-four-hour urine samples were collected sequentially before the initiation of therapy, during the intra-arterial infusion of doxorubicin, and during radiation therapy. Samples were preserved with 0.05% sodium azide at 4°C and processed immediately after collection. Samples were filtered and concentrated (100-fold) with a hollow fiber concentrator and a PM-10 membrane (molecular weight, 10,000) pressure filtration cell (Amicon). The concentrate was dialyzed at 4°C against 0.025 M phosphate buffer (pH 7.4) supplemented with 0.85% NaCl (phosphate-buffered saline) for 18 hours. Samples were stored at –35°C until testing. An aliquot of unprocessed urine was analyzed for creatinine content. Final results of antigen determinations were standardized against the creatinine content of each specimen to correct for day-to-day fluctuations in urine volume.

TABLE 1.—Histologic classification of primary tumor

Histology	No. of patients
Osteosarcoma	15
Liposarcoma	8
Fibrosarcoma	6
Leiomyosarcoma	5
Chondrosarcoma	4
Fibrous histiocytoma	4
Rhabdomyosarcoma	3
Anaplastic sarcoma	3
Ewing's sarcoma	2
Synovial cell sarcoma	2
Lymphangiosarcoma	1

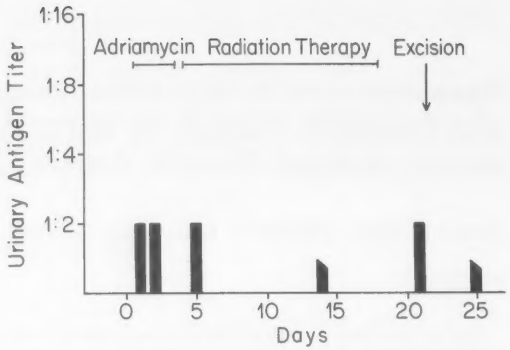

FIGURE 1.—Preoperative sarcoma treatment protocol. Following biopsy of the primary tumor, doxorubicin (30 mg/day × 3 days) was infused through the major artery feeding the tumor. Radiation therapy (350 rads/day) was administered in 10 fractions. Unless otherwise indicated, the radical excision of the tumor was performed 7 days following completion of therapy.

Antibody Source

Autologous and allogeneic sarcoma sera were used as the sources of specific antibody in the complement fixation assay. The autologous serum sample was collected before the initiation of therapy. The allogeneic serum sample had been shown to have a high complement-fixing antibody titer to sarcoma cells and its specificity was previously established by absorption studies (6,8). A normal human serum known to have no antibody activity against sarcoma cells was used as a negative control. The urinary antigen titers reported in this study are against the allogeneic sarcoma serum. This serum sample was obtained from a patient with sarcoma.

Complement Fixation Assay

Antigenic activity in the urine samples was assessed by the ultramicrocomplement fixation assay described by Gupta and Morton (5). Two microliters each of appropriately diluted urine, serum, and human umbilical cord serum (complement source) was injected and mixed in the wells of a Terasaki tissue culture plate (Falcon Plastics, Oxnard, CA) filled with mineral oil of light density. The plates were incubated for 1 hour at 37°C and were then inoculated with 2 μl of 0.5% sensitized sheep rbcs (Flow Laboratories, Rockville, MD). After a 30-minute incubation period at 37°C, the plates were read visually over a serologic laboratory view light. A titer was defined as the highest dilution of urine showing ≤ 40% hemolysis of the sheep rbcs. Simultaneous

internal controls were run for anticomplementary activity of the serum and urine as well as for the sensitivity of the sheep cells. A test sample was considered positive for antigen when reactivity against antiserum was present at a urine dilution ≥ 1:16. Urine samples that exhibited anticomplementary activity were considered antigen-positive only when the titer was at least two dilutions above the anticomplementary activity. The few samples in which anticomplementarity equaled antigen titer were excluded, since antigen titer in these instances could not be interpreted. A twofold (one dilution) change in antigen titer was found to be within the experimental variation of the complement fixation assay, and thus only a fourfold or greater change in antigen titer was considered significant.

RESULTS

Of the 53 consecutively treated patients who received the preoperative therapy, the urine samples of 44 exhibited a fourfold or greater increase in antigen titer (range, 4–128) during the treatment period compared to the pretreatment antigen level. A representative profile of consecutive antigen titers is shown in figure 2. In general, antigen titers rose sharply within 12–24 hours after the initiation of doxorubicin infusion. This titer then decreased with the cessation of doxorubicin infusion. The titer increased again 4–6 days after the initiation of radiation therapy. Following resection, antigen titers usually decreased within 2 weeks. For the patient whose titers are shown in figure 2, excision of tumor was delayed for > 21 days, because the femur prosthesis was not available on Day 21.

Table 2 shows that all of the 44 patients who had an increase in antigen titer during the treatment period had evidence for in situ tumor cell destruction. Thirty-two patients had 20%–100% necrosis in the resected specimen. Five had complete resolution of palpable tumor nodules (confirmed tumor by preoperative incisional biopsy), and seven were known to have positive margins following pretreatment biopsy. These 12 patients had no viable tumor detectable in the resected specimen, indicating com-

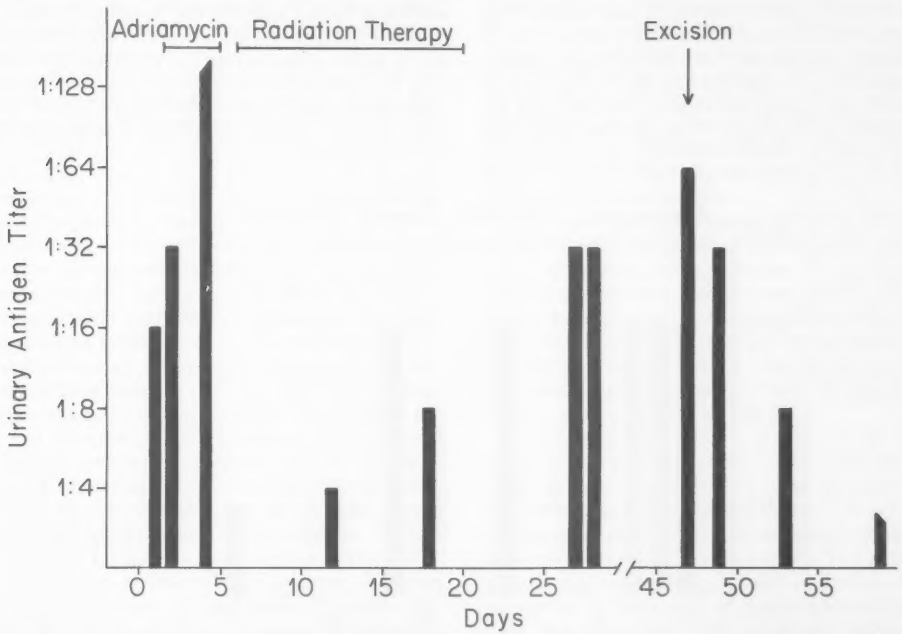

FIGURE 2.—Urinary antigen profile of a 17-yr-old female with an osteosarcoma of the femur, showing response to therapy. Prior to treatment, antigen was detectable in the urine at a dilution of 1:16. Antigen titers increased during doxorubicin infusion and again during radiation therapy, but decreased to nonsignificant levels (< 1:16) after resection. Resected tumor specimen was almost completely (96%) necrotic.

TABLE 2.—Comparison of the change of urinary antigen titer with the clinical and pathologic tumor response to doxorubicin and radiation therapy

Criteria for effectiveness of therapy	No. of patients	No. exhibiting ≥ 4-fold increase in titer
> 20% necrosis	32	32
Disappearance of documented residual tumor	7	7
Disappearance of palpable tumor nodule	5	5
< 5% necrosis (ineffective therapy)	7	0
Clear margin prior to initiation of therapy	2	0

plete destruction of the residual tumor by the preoperative therapy.

Nine patients had no demonstrable rise in urinary antigen titer during the preoperative treatment period. Two of these patients had excisional biopsies with clear margins prior to therapy. These patients underwent preoperative treatment and re-resection of the tumor bed because of proximity of the tumor to the biopsy margin and because of tumor grade. The remaining seven patients in this group had no evidence of significant necrosis (< 5%) induced by the preoperative therapy. Thus, in the seven patients, the failure to detect an increase in urinary antigen levels during the treatment period reflected failure of the preoperative therapy. Representative antigen profiles of nonresponding patients are shown in figures 3 and 4.

DISCUSSION

Analysis of urinary antigen titers in 44 patients receiving preoperative treatment of doxorubicin and radiation therapy demonstrated a strong correlation between an increase in antigen titer and clinicopathologic evidence of tumor cell destruction. The resected tumor specimens from 32 patients had histologic evidence of cellular destruction, ie, loss of nuclear detail with hematoxylin and eosin stain. The remaining 12 patients in this group had total destruction of tumor by the preoperative therapy.

The nine patients who had no increase in antigen titer during the treatment period can be categorized into two subgroups. The first subgroup was composed of the two patients who had complete excisional biopsies before treatment. Both patients were placed on the preoperative protocol because of insufficient biopsy margins and histologic tumor grade. Neither patient had significant levels of antigen activity in the urine before the initiation of treatment (fig 4). The seven patients whose tumors did not respond to the doxorubicin and radiation therapy were in the second subgroup. Comparison of the

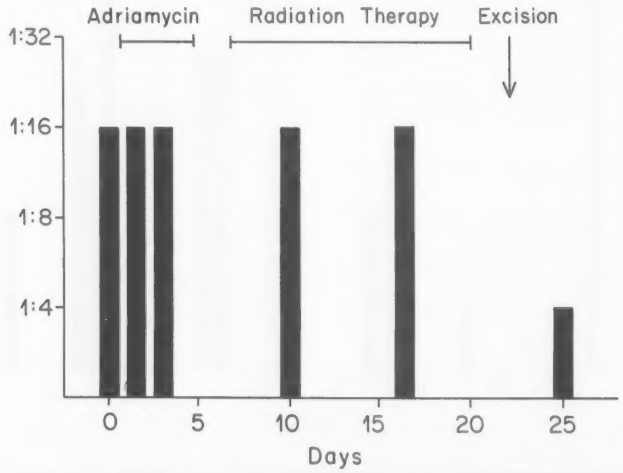

FIGURE 3.—Antigen profile of a nonresponding 19-yr-old male with a fibrosarcoma of the distal leg. No significant change in antigen titer occurs during the treatment period. Antigen disappears following resection. Tumor necrosis was estimated to be 5% in both the pretreatment biopsy and the resection specimen.

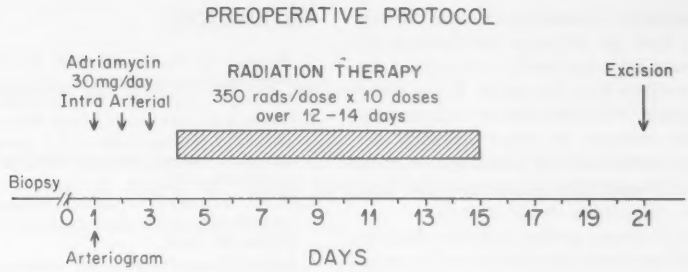

FIGURE 4.—Antigen profile in tumor-free patient. Pretreatment biopsy completely removed this fibrosarcoma. However, the tumor bed was treated because of tumor grade and proximity of tumor to the margin. No significant amount of antigen is detectable at any time in this patient's urine.

pretreatment biopsy specimens and the posttreatment operative specimens showed < 5% necrosis in both specimens. The failure to demonstrate a rise in antigen titer could not be attributed to differences in tumor antigenicity, since the pretreatment antigen titers in the nonresponding patients compared favorably to the titers of patients in the responding group. Furthermore, both the nonresponders and responders had similar histologic types and grades of tumors.

The possibility that decreased urinary antigen titers observed after tumor excision could be due to increased anticomplementary activity of the urine is unlikely. Urine was considered antigen-positive when the titer was at least fourfold above the anticomplementary activity and the final dilution showing the positive reaction against the antibody was considered as the antigen titer. The urine samples that were anticomplementary and did not fulfill the above criteria were excluded from the study.

The antitumor antibody properties of the allogeneic antisera used for these determinations have been demonstrated in previous reports (6,8). Absorption with various normal human tissues failed to reduce antibody activity against the urinary antigen, but absorption with sarcoma cells prepared from biopsy specimens or tissue culture cell lines completely absorbed antibody activity. To preclude antibodies to doxorubicin, the autologous serum samples were collected before the initiation of therapy. A control for non-tumor-specific activity in the urine is the use of normal serum from a human subject with no history of malignancy. Because nine patients did not develop any increase in urine activity during the administration of doxorubicin and radiation therapy, the possibility of nonspecific effects of those treatment modalities as a cause for the observed results is discounted.

The allogeneic serum used as a positive control was obtained from a patient with sarcoma who demonstrated high antibody titer throughout a period of several months. This serum was chosen because of its high titer. However, to avoid misinterpretation of results due to reactivity against histocompatibility antigens, each patient's urine was also tested against autologous serum obtained prior to the start of therapy. Each serum sample demonstrated some antibody activity, but the absolute level varied from patient to patient. In addition, serum samples from the same patient varied with respect to antibody activity during the course of treatment. Therefore, one allogeneic serum sample from a patient with sarcoma was used throughout to maintain the consistency of results.

The reason for wide cross-reactivity is not clear. However, this study only examines reactivity in the urine of patients with sarcoma. Thus, despite the variety of histologic tumor types, all have a common embryologic precursor, the mesoderm. Whether this is the basis for the cross-reactivity is unknown, and further studies are under way involving patients with soft tissue trauma and autoimmune diseases such as rheumatoid arthritis.

In this group of patients, we have been unable to detect consistent differences in the level of antigen titers among the various histologic types of tumor. Furthermore, the absolute amount of antigen detected does not necessarily correlate with the estimated amount of tumor necrosis. The antigen has a high molecular weight, and excretion does not appear to be the result of simple glomerular filtration. We have been able to detect immune complexes in the serum of cancer patients and correlate the level of these complexes with the level of urinary antigen. In addition, we have detected immune complex deposition in the glomerular basement mem-

brane of some patients (unpublished data). These findings suggest that an immune mechanism involving antigen and host antibody is required for the excretion of antigen into the urine. Thus, urine antigen levels appear to depend on the antigenicity of the tumor, the amount of tumor actually destroyed, and the amount of circulating antibody available to bind to the antigen resulting in the deposition of immune complexes in the kidney.

The results of this investigation indicate that serial assessment of the levels of antigen in the urine of patients with sarcoma who are receiving potentially tumoricidal therapy can be used to indicate the effectiveness of the treatment. It would appear that membrane-bound and cytoplasmic antigens are released from the tumor into the bloodstream during cell death. These antigens subsequently pass into the urine and can be detected by immunologic means.

In the future, this phenomenon could be used to measure the in vivo response of nonaccessible tumors to tumoricidal therapy.

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Pharmacokinetics of Vincristine Infusion [1,2]

Don V. Jackson, Jr,[3,*] V. Sagar Sethi,[3] Charles L. Spurr,[3] Douglas R. White,[3] Frederick Richards, II,[3] John J. Stuart,[3] Hyman B. Muss,[3] M. Robert Cooper,[3] and Manford C. Castle[4,5]

SUMMARY

Using a sensitive radioimmunoassay, serial blood concentrations of vincristine were measured in 11 patients with refractory malignancies receiving infusions of vincristine at three dose levels: 0.5, 0.75, and 1.0 mg/m² daily for 5 days. The pharmacokinetics of vincristine infusion were compared to pharmacologic data obtained from four patients who received conventional iv bolus injections of 2 mg of vincristine. Whereas rapid decline of blood levels was seen following iv bolus injection, with concentrations of vincristine approaching 10^{-9} M by 48–72 hours, vincristine infusions of 0.5, 0.75, and 1.0 mg/m² daily for 5 days consistently resulted in blood concentrations $> 10^{-9}$ M during the treatment period. Similarly, areas under the concentration curve were greater in patients receiving infusions compared to bolus injections of vincristine. Antitumor responses in patients receiving vincristine infusions were observed after failure to respond to iv bolus injections. These data demonstrate the ability of infusion therapy to sustain blood concentrations of vincristine in man beyond that seen with conventional administration and suggest the possibility of improved therapeutic efficacy with this agent by use of infusion techniques.

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Vincristine, an antimitotic alkaloid derived from the plant *Vinca rosea* Linn, has been widely employed in cancer chemotherapy for nearly two decades, using iv bolus injection (1,2). However, we and others (1,3–5) have found a rapid disappearance of the drug from the blood following this method of administration. Furthermore, in vitro studies have demonstrated the antitumor effect of this agent to be critically dependent on both concentration and duration of exposure (6). These data suggested that infusion techniques might sustain levels of vincristine and enhance its therapeutic efficacy (6). The pharmacokinetics of vincristine have been analyzed during a phase I trial (7) using a 5-day continuous infusion of this agent.

MATERIALS AND METHODS

Patients

From October 1979 to May 1980, 30 patients with malignancies refractory to conventional treatment were entered in a phase I trial of vincristine infusion (7); 11 underwent pharmacologic evaluation at the Bowman Gray School of Medicine of Wake Forest University. Patient characteristics are listed in table 1.

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[3]Oncology Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

[4]Department of Pharmacology, Eastern Virginia Medical School, Norfolk.

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**Reprint requests to:* Don V. Jackson, Jr, MD, Oncology Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

TABLE 1.—Patient characteristics

Dose	Patient No.	Age (yrs)	Sex	Diagnosis	Body surface area (m^2)
0.5 mg/m^2/day infusion	1*	60	M	Lymphoma	1.9
	2	42	F	Lymphoma	1.6
	3	48	M	Lymphoma	1.8
	4	67	M	Lymphoma	1.6
0.75 mg/m^2/day infusion	5	34	M	Leukemia	2.0
	6	34	M	Leukemia	1.7
	7	44	M	Leukemia	1.9
	1*	60	M	Lymphoma	1.9
1.0 mg/m^2/day infusion	8	25	M	Leukemia	1.9
	9	38	F	Breast cancer	1.5
	10	61	F	Breast cancer	1.4
	11	65	F	Breast cancer	1.8
2.0 mg bolus	12	57	F	Colon cancer	1.7
	1*	60	M	Lymphoma	1.9
	13	61	M	Lymphoma	1.9
	14	63	M	Hepatoma	1.8

*Pharmacokinetic analyses were performed for patient 1 after vincristine iv bolus injection and after infusions of 0.50 and 0.75 mg/m^2/day × 5. Single determinations were done for all other patients.

Infusion

Patients were administered vincristine (Eli Lilly and Co, Indianapolis, IN) at a dose of 0.5 mg by iv bolus injection to rapidly achieve potentially cytotoxic blood levels. Following this, a continuous infusion of vincristine by iv drip was begun at one of three dose levels: 0.5, 0.75, or 1.0 mg/m^2 daily for 5 days. The infusate consisted of 1000 ml of 5% dextrose in water to which was added the appropriate daily dose of vincristine, 3000 units of heparin, and 50 mg of hydrocortisone to reduce phlebitis.

The stability of vincristine under the above conditions was estimated by a modification of the high-pressure liquid chromatographic method described previously (8,9). In the revised procedure, 10 ml of a typical infusate (containing 1.0 mg of vincristine/liter) was pumped (2.5 ml/minute) into a LiChrosorb C_{18} reversed-phase column (E. Merck Co, Elmsford, NY). This permitted the vincristine and any decomposition products to be deposited at the front of the column. Vincristine and its degradation products were eluted from the column using a linear gradient (at 5%/minute) consisting of 0.001 M K_2HPO_4 buffer as solvent A and 80% acetonitrile in water (which also contained K_2PO_4 at a concentration of 0.001 M) as solvent B. Results from these studies using both ^{3}H-vincristine (Amersham Corp, Arlington Heights, IL) and nonradiolabeled vincristine suggested that decomposition of vincristine over a 24-hour period was < 10%.

Samples

Serial serum samples were obtained by removal of 3 ml of whole blood from the arm opposite the injection or in the same arm below the site of administration. A 23-gauge scalp vein needle was attached to a heparin lock (Abbott Co, Chicago, IL) from which blood samples were removed during the first 12–24 hours following vincristine administration; venipuncture was used to obtain samples at later time points. Blood samples were centrifuged, and the resultant serum was stored at –20°C until processed.

The total voided urine from two patients was collected in containers cooled with ice. After recording the total daily volume, an aliquot was obtained and stored at –20°C until processed.

Radioimmunoassay

Serum and urine samples have been analyzed by modification of the radioimmunoassay described by Owellen et al (4), which has allowed a fivefold to sevenfold greater sensitivity to ~5×10^{-10}–10^{-9} M (10). Vincristine, lot 67522, provided by Dr. John Douros, National Cancer Institute, Bethesda, MD, was used as a standard. Tritium-labeled vinblastine (specific activity, 10.4 curies/mmol) was obtained from Amersham Corp. The unfractionated lyophilized antiserum, obtained from rabbits by injection of 4-deacetylvinblastine C-3 carboxazide coupled to

bovine serum albumin, was provided by Dr. Mary Root, Lilly Research Laboratories, Indianapolis, IN. Appropriate dilutions of the serum or urine samples were made in the glycine buffer (0.2 M glycine-HCl, pH 8.8; 0.25% crystalline human albumin [ICN Pharmaceuticals, Inc, Cleveland, OH]; 1% normal lamb serum [North American Biologicals, Inc, Miami, FL]; and 242 mg of merthiolate/liter). In duplicate glass test tubes, 100 μl of the known quantity of the standard alkaloid solution (or various dilutions of the serum or urine samples), 100 μl of the diluted antiserum or normal rabbit serum, and 100 μl of the glycine buffer were incubated for 60 minutes at 4°C in enclosed plastic containers, after which 100 μl of 1:2400-diluted ^{3}H-vinblastine in the glycine buffer was added to each tube. The reaction mixtures were allowed to incubate for another 60 minutes in the refrigerator. At the end of the incubation period, 400 μl of well-stirred dextran-coated charcoal suspension (1% carbon decolorizing [Fisher Scientific Co, Fairlawn, NJ] and 0.5% dextran 70 [Pharmacia Fine Chemicals Co, Uppsala, Sweden] suspended in the glycine buffer) was added to each tube, and the contents were mixed thoroughly, kept at room temperature for 20 minutes, and centrifuged at 2700 rpm for 10 minutes. Five hundred microliters of the clear supernatant from each tube was mixed thoroughly with 4 ml of toluene-PPO-POPOP[6]:Triton X-100 (2:1) scintillation fluid, and the radioactivity was counted against an external standard in a liquid scintillation counter. Radioactivity from each sample was converted into dpm from a standard quench curve. From the total amount of input radioactivity, percent labeled alkaloid bound was calculated after subtracting the nonspecific radioactivity. The nonspecific binding of the ^{3}H-vinblastine or ^{3}H-vincristine was between 1% and 3% of the total input radioactivity. For these calculations a computer program was set up which converted the raw cpm data into dpm, took an average of the duplicate values, subtracted the background, and calculated the percent bound radioactivity. From the known amount of vincristine and percent bound radioactivity, a standard curve for each experiment was plotted on a log-logit graph from which the amount of vincristine present in each diluted blood or urine sample was determined. Vincristine concentrations closest to 50% competition of binding were taken as final values. The 50% competition level of vincristine from 30 standard curves was 0.27 ± 0.082 ng (mean ± SD). The results are expressed as concentrations of vincristine which refer to vincristine equivalents (parent drug and its metabolic and/or decomposition products detected by radioimmunoassay).

[6]PPO-POPOP = 2,5-phenyloxazol-2,2′-*p*-phenylene-bis(4-methyl-5-phenoxazol).

Pharmacokinetic Analyses

Four patients receiving conventional 2.0-mg iv bolus injections of vincristine and four patients at each vincristine infusion dose level underwent pharmacokinetic evaluation and provide the basis for this report. Area under the concentration curve (AUC) from Time 0 to infinity and total-body clearance have been determined by conventional methods (11) in patients receiving iv bolus injections. To determine the AUC of patients undergoing infusion, a paper-weighing method was used. After plotting drug kinetic data on smooth linear graph paper, the paper was cut and weighed on an analytic balance. Finally, AUC data for infusion were calculated using a conversion factor of 46.91 nM × minute/mg paper weight which had been derived from the data obtained in patients who had received iv bolus injections. The contribution of the AUC obtained following discontinuation of infusion was determined in two patients and was found to be 14.7% of the AUC from Time 0 to 120 hours. This value was incorporated into the AUC determinations of the remaining patients to allow determination of concentration × time values from 0 to infinity.

RESULTS

Steady-state blood concentrations > 10^{-9} M have been found during each of the infusion dose levels employed in the current trial (fig 1 and table 2). Following infusion of 0.5 mg/m^2 of vincristine daily for 5 days, 24–120-hour serum vincristine concentrations ranged between 2.1 × 10^{-9} and 1.3 × 10^{-8} M in four patients examined. Urinary excretion from two of these patients accounted for 28.4% and 21.6% of the total administered dose over a 5- and 6-day period of observation, respectively. At the 0.75-mg/m^2 dose level, 24–120-hour vincristine concentrations ranged between 1.1 × 10^{-9} and 4.8 × 10^{-9} M during the 5-day period of infusion in four patients. Serum vincristine concentrations between 1.9 × 10^{-9} and 1.0 × 10^{-8} M at 24–120 hours of infusion have been attained in four patients receiving 1.0 mg/m^2 of vincristine daily for 5 days.

Serial blood samples were obtained from two patients during a 24-hour period after discontinuation of the 5-day infusions. Half-lives following infusion doses of 0.5 and 1.0 mg/m^2 were 21.7 and 10.5 hours, respectively, which are similar to the terminal half-lives observed in the four patients receiving iv bolus injections of vincristine only (10.9–24.7 hours).

TABLE 2.—Pharmacokinetic parameters*

Patient No.	Loading dose (mg)	Infusion dose (mg/m^2/day)	K_0 (nanomols/min)	C^{ss} (nM)	AUC (nM × mins)	TBC (ml/min)
1	0.5	0.50	0.678	2.33 ± 0.12	32,821	290.4
2	0.5	0.50	0.602	4.41 ± 0.23	58,043	136.4
3	0.5	0.50	0.677	3.75 ± 0.12	43,420	180.5
4	0.5	0.50	0.602	11.20 ± 2.53	81,838	53.7
5	0.5	0.75	1.128	2.11 ± 0.17	27,333	534.6
6	0.5	0.75	0.977	3.72 ± 0.57	37,762	262.6
7	0.5	0.75	1.052	2.45 ± 0.69	25,814	429.3
1	0.5	0.75	1.052	2.80 ± 0.60	35,618	375.6
8	0.5	1.0	1.421	2.08 ± 1.24	27,459	683.2
9	0.5	1.0	1.128	3.14 ± 1.13	44,120	359.2
10	0.5	1.0	1.068	8.56 ± 4.35	75,704	124.7
11	0.5	1.0	1.324	6.52 ± 2.23	59,078	203.0
1	2.0	—	—	—	19,045	177.4
13	2.0	—	—	—	9966	100.4
12	2.0	—	—	—	17,973	190.2
14	2.0	—	—	—	25,257	189.0

*K_0 = rate of infusion; C^{ss} = steady-state concentration of vincristine equivalents in the serum obtained by averaging the 48-, 72-, 96-, and 120-hr values for each patient (± SD); TBC = total-body clearance.

In figure 1, serial mean serum vincristine concentrations of four patients at each infusion dose level are compared to the mean serum vincristine concentrations of four patients receiving 2 mg of vincristine by iv bolus injection. The triexponential decay curve inscribed following conventional iv bolus injection shows a rapid disappearance of vincristine from the blood (α-half-life = 2.9 ± 0.6 minutes and β-half-life = 23.7 ± 8.5 minutes) with concentrations approximating 10^{-9} M by 48–72 hours (γ-half-life = 18.0 ± 5.9 hours), whereas infusions at each of the three dose levels resulted in vincristine concentrations $> 10^{-9}$ M during the entire treatment period (5 days). Similarly, the AUC following infusions were greater than those generated after bolus injection of 2.0 mg of vincristine (table 2). Steady-state serum levels during infusions at the same dose were variable. As in the patients receiving conventional iv bolus injections of vincristine, there appeared to be a great deal of individual variation in the patients receiving infusions of vincristine with respect to total-body clearance and AUC (table 2). The wide range of differences in these parameters during infusion could not be explained by dissimilarities in pretreatment characteristics or hepatic and renal functions.

DISCUSSION

Using a clonogenic assay, 50% cell-kill of L1210 murine leukemia has been found following a 3-hour period of exposure to 10^{-7} M vincristine, whereas a 6–12-hour period of exposure was required to achieve this degree of cytotoxicity with 10^{-8} M vincristine (6). Continuous in vitro exposure of this cell line and CEM human lymphoblastoid leukemia to 10^{-9} M vincristine for periods of ≥ 24 hours resulted in no appreciable cell death (6). These in vitro data suggested the potential for improved therapeutic efficacy of this drug in man by using techniques designed to achieve and sustain blood concentrations of vincristine at $> 10^{-9}$ M.

Vincristine concentrations of 10^{-7}–10^{-8} M are clinically attainable in the blood following iv bolus injection as illustrated in the four patients presented herein. However, serum concentrations decrease to levels $< 10^{-8}$ M by 2–4 hours after injection and approach 10^{-9} M by 48–72 hours. The prolonged low concentrations of this agent following conventional administration probably are a result of avid tissue binding of vincristine from which a slow release occurs (1,4,5).

Infusion of vincristine, however, in the doses employed in this study (0.5, 0.75, and 1.0 mg/m^2 daily), has consistently resulted in the attainment of steady-state blood levels of vincristine at $> 10^{-9}$ M during the 5-day treatment period. Furthermore, greater drug concentration × time measurements (AUC) have been observed following vincristine infusions of 0.5, 0.75, and 1.0 mg/m^2 daily for 5 days compared to iv bolus injections of approximately 1.0–1.2 mg/m^2 of vincristine. Blood levels of vinblastine, a congener of vincristine, during daily infusions of ≥ 1.0 mg/m^2 have also been reported to be 10^{-8}–10^{-9} M (12,13). The disappearance of vincristine from the blood following discontinuation of infusion (half-life, 10.5–21.7 hours) is also similar to that determined

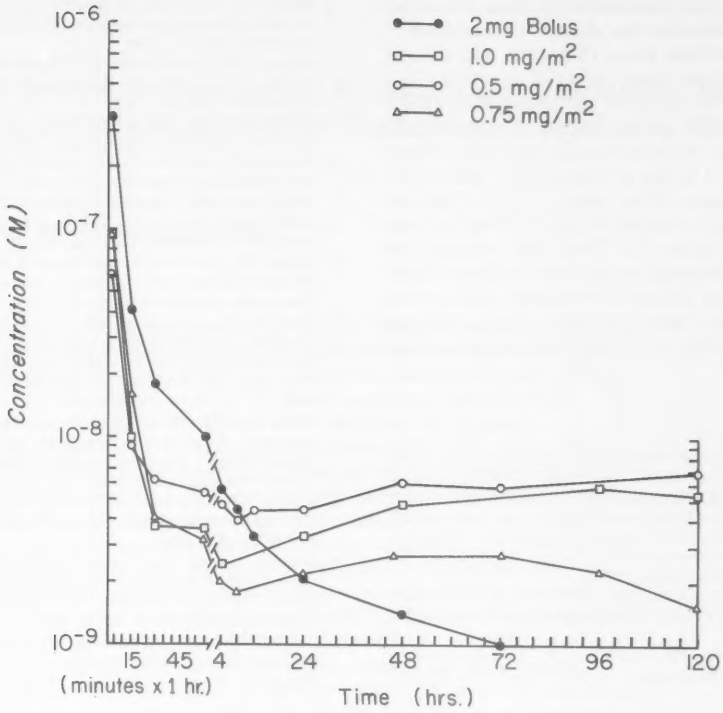

FIGURE 1.—Comparison of mean serum concentrations of vincristine and its metabolic and decomposition products following iv bolus injection or infusion. Each curve is generated from data obtained in 4 patients.

by radioimmunoassay following completion of vinblastine infusion (half-life, 19 hours) (13).

The clinical results of this trial have been discussed elsewhere (7). Neurotoxicity was the principal side effect and was severe with the high infusion dose. Most patients who demonstrated an objective response to infusion of vincristine had previously received vincristine administered by iv bolus injection (7). The ability to respond to infusion techniques following failure of iv bolus injection has also been reported for two other closely related vinca alkaloid derivatives: vinblastine (12) and vindesine (14). The development of toxicity or response in our trial (7) could not be predicted by inspection of the pharmacologic parameters observed in the present report.

The wide range of pharmacokinetic parameters observed among the different infusion dose groups may be due to individual variability in hepatic disposition of the drug since the biliary system appears to be the major route of excretion of vincristine and its products in man (8). Increased biliary excretion of vincristine and its products as observed in rats given increasing doses of vincristine (15) may account for the inability to attain a stepwise increase in steady-state vincristine concentrations in the blood with increasing infusion dose.

Except in one patient (No. 1), the total amount of vincristine given to each of the patients during these infusion studies has exceeded the usual individual iv bolus dose (2 mg). On one occasion this patient received a total vincristine dose of 1.9 mg during a 3-day period of infusion, which resulted in a greater AUC (24,618 nM × minute) than had been observed following a 2-mg bolus injection of vincristine 7 months earlier (19,045 nM × minute). This observation and the finding of similar ranges of steady-state blood concentrations of vincristine, despite a wide range of infusion dose, may indicate the ability to produce similar pharmacologic results with less host toxicity by using lower infusion doses than those investigated in the present study.

In summary, the pharmacologic data presented in this report establish the ability of vincristine iv infusion at three dose levels (0.5, 0.75, and 1.0 mg/m^2 daily) to produce blood levels of vincristine in a range found to be cytotoxic for mammalian tumor cells in vitro (>10^{-9} M) (6). Such levels have been sustained for time periods beyond that seen with iv bolus injection of 2 mg of vincristine. Moreover, antitumor responses have been noted in patients eventually failing to respond to conventional iv bolus injection of this agent (7). These data suggest the possibility of enhancing the antitumor effects of vincristine in man by infusion techniques and provide the pharmacologic basis for further exploration of this promising method of vincristine administration.

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Increased Schedule-Dependent Synergism of Vindesine Versus Vincristine in Combination With Methotrexate Against L1210 Leukemia[1,2]

Paul L. Chello* and Francis M. Sirotnak[3]

SUMMARY

$BD2F_1$ mice were inoculated ip with 10^6 L1210 leukemia cells and treated with vincristine or vindesine alone or in combination with methotrexate. Drug administration was begun on Day 1 and was continued every 4 days until a total of five doses were given or death occurred. Methotrexate (48 or 72 mg/kg ip) produced a 199% and a 222% increase in lifespan, respectively, as compared with untreated animals (6.9 ± 0.5 days). When given as single agents, vincristine (0.5–1.0 mg/kg ip) or vindesine (0.5–1.5 mg/kg ip) produced between a 27% and an 88% increase in lifespan. The therapeutic benefit observed when either vinca alkaloid was used with methotrexate was schedule-dependent. With the exception of vindesine plus 72 mg/kg of methotrexate, the increase in lifespan produced by the simultaneous administration of methotrexate and either vinca alkaloid was additive. When vindesine was administered with 72 mg/kg of methotrexate, the increase in lifespan was greater than expected from an additive effect of the two agents. However, none of the trials employing single-agent therapy or simultaneous combination therapy produced long-term survivors (≥ 90 days after therapy). When either vinca alkaloid was given 24 hours after the folate analog, the increase in lifespan was almost 100% greater than that observed when the agents were given simultaneously; moreover, long-term survivors were produced. Vindesine in combination with 48 mg/kg of methotrexate produced 10%–25% long-term survivors, as compared to 5%–7% long-term survivors obtained with vincristine. In combination with 72 mg/kg of methotrexate, vindesine produced 27%–60% long-term survivors, as compared to 10%–20% long-term survivors obtained with vincristine. When either vinca alkaloid was administered 72 hours after methotrexate, the regimen was still synergistic, but the overall effect was less than with a 24-hour delay. When two doses of either vinca alkaloid were injected at 24 and at 72 hours after the folate analog, the result was either highly therapeutic or very toxic. Two doses of 0.5 mg/kg of vindesine or vincristine with 48 mg/kg of methotrexate produced 35% and 20% long-term survivors, respectively. All other regimens were toxic.

[Cancer Treat Rep 65:1049–1053, 1981]

The therapeutic effect when vincristine is used with methotrexate for the treatment of mice bearing L1210 leukemia is schedule-dependent. In contrast to an earlier report (1), we have shown (2,3) that the administration of vincristine prior to or together with methotrexate produces either no increase in therapeutic effect over that seen with methotrexate alone, or a therapeutic gain no greater than expected from an additive effect of the two

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[3]Laboratory for Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center, New York, NY.

*Reprint requests to: Paul L. Chello, PhD, Laboratory for Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

agents. However, a delay in administration of the vinca alkaloid for at least 24 hours after the folate analog can produce an additional 100% increase in lifespan and can result in long-term survivors (≥ 90 days after treatment) (2,3). The biochemical basis for the synergism is unknown. Studies from this laboratory (2,3) and elsewhere (4) have shown that it is not due to an increase in the intracellular level of exchangeable methotrexate in tumor cells in vivo, as originally suggested by in vitro studies (1,5,6).

Vindesine is a promising analog of vinblastine currently in phase II trial (7–13). It binds as effectively to tubulin in vitro as does vincristine or vinblastine (14,15), has a broader spectrum of activity against animal tumors than either agent (16), and is less neurotoxic than vincristine (17).

Because the synergism between methotrexate and vincristine may suggest a better clinical scheduling of these two agents, and because the increased spectrum of activity and reduced toxicity of vindesine as compared to vincristine makes it a potentially more useful agent, we undertook a comparative evaluation of the therapeutic effectiveness of vindesine versus vincristine, plus methotrexate, against L1210 leukemia. The two vinca alkaloids were compared simultaneously on the best schedules already delineated with vincristine (3). The results were very encouraging. In practically every instance, vindesine was more effective than vincristine, and the lesser toxicity observed with vindesine also permitted a higher level to be tested in combination with methotrexate.

MATERIALS AND METHODS

Vincristine (Oncovin) and vindesine were from Eli Lilly and Co, Indianapolis, IN. Methotrexate (sodium salt) was provided by Dr. Harry B. Wood, Jr, Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. All drugs were prepared in sterile distilled water. L1210 tumor cells (line V) were maintained (18) by ip transplantation in female $B6D2F_1$ mice [(57BL/6 × DBA/2)F_1] obtained from A. R. S. Sprague/Dawley, Madison, WI. For therapy experiments, female mice (initial weight, 20.0 ± 0.5 g) received 10^6 L1210 tumor cells ip on Day 0 and were randomly distributed between control (untreated) and treated groups. Untreated animals died with well-developed ascites within 6–7 days. Treated animals received each drug separately by ip injection beginning 24 hours (Day 1) after inoculation with tumor cells. All animals were maintained with Purina laboratory chow and water ad libitum.

RESULTS AND DISCUSSION

Since our previous study (3) had shown that five doses of methotrexate given every 4 days followed by vincristine at 24, 72, or 24 and 72 hours represented the most effective use of these agents for treatment of L1210 leukemia, this schedule was chosen for the present study. Although the study (2,3) also indicated that the therapeutic gain after simultaneous administration of the two agents represented only an additive effect, this schedule was included in the present study for comparison purposes. All doses were selected from probit analyses of survival data of normal mice following ip administration of individual drugs every 4 days, for a total of five doses. The LD10 and LD50 values for vincristine were 0.66 and 1.3 mg/kg, respectively; the comparable values for vindesine were 1.12 and 2.1 mg/kg, respectively. The LD10 and LD50 values for methotrexate were 75 and 165 mg/kg, respectively. The survival data presented in tables 1 and 2 for untreated animals and for animals treated with either vinca alkaloid given as a single agent relate to the experiments presented in that particular table.

The therapeutic effect of the vinca alkaloids alone and in combination with 48 mg/kg of methotrexate is shown in table 1. Control animals survived an average of 6.9 ± 0.5 days. When administered as single agents, neither vincristine nor vindesine was comparable therapeutically to methotrexate. However, vindesine was more effective against L1210 leukemia in this schedule than previously reported for a daily schedule (16). When either of the vinca alkaloids was administered together with methotrexate, the increase in lifespan normally fell within a range which could be explained by an additive effect of the two agents. Vindesine was not superior to vincristine when used in this manner.

When the vinca alkaloids were administered 24 hours after the folate analog, the two agents interacted in a synergistic manner. In some cases, the lifespan was extended by almost 100% beyond that observed when the agents were used together. More importantly, the delay of vincristine or vindesine administration resulted in long-term survivors, ie, animals surviving ≥ 90 days after treatment with no evidence of tumor. No long-term survivors ever resulted from single-agent therapy or from therapy with the agents given at the same time. The administration of vindesine 24 hours after methotrexate produced a slightly greater increase in lifespan than that observed with vincristine. Vindesine at doses of 0.5 and 1.0 mg/kg produced 10% and 15% long-term survivors, respectively. However, these survival rates were not statistically different ($P > 0.05$)

TABLE 1.—Treatment of L1210 leukemia* with 48 mg/kg of methotrexate (MTX) ip and vincristine (VCR) ip or vindesine (VDS) ip

MTX† (mg/kg)	VCR (mg/kg)	VDS (mg/kg)	Interval between MTX and vinca alkaloid therapy	Total No. of mice‡	MST ± SD§ (days)	Increase in lifespan (%)	Final weight ± SD¶ (g)	90-day survivors**
—††	—	—	—	5 × 5	6.9 ± 0.5	—	21.1 ± 0.7	0/25
—	0.5‡‡	—	—	5 × 5	9.2 ± 1.3	33	20.7 ± 1.2	0/25
—	1.0‡‡	—	—	3 × 5	11.6 ± 1.5	68	18.2 ± 1.2	0/15
—	—	0.5‡‡	—	5 × 5	8.8 ± 1.0	27	21.0 ± 1.2	0/25
—	—	1.0‡‡	—	5 × 5	10.4 ± 1.2	50	19.6 ± 1.3	0/25
—	—	1.5‡‡	—	5 × 5	13.0 ± 3.3	88	18.4 ± 1.7	0/25
48	—	—	—	5 × 5	20.6 ± 3.7	199	20.7 ± 0.9	0/25
48	0.5	—	Together	4 × 5	21.5 ± 8.2	211	18.3 ± 2.6	0/20
48	1.0	—	Together	3 × 5	24.9 ± 3.8	261	19.9 ± 3.3	0/15
48	—	0.5	Together	3 × 5	24.4 ± 3.1	253	18.7 ± 1.4	0/15
48	—	1.0	Together	3 × 5	25.3 ± 6.2	267	18.7 ± 2.6	0/15
48	—	1.5	Together	3 × 5	26.5 ± 8.4	285	20.4 ± 2.1	0/15
48	0.5	—	24 hrs	4 × 5	28.0 ± 6.6	306	16.3 ± 5.0	1/20
48	1.0	—	24 hrs	3 × 5	31.1 ± 10.7	350	17.5 ± 3.1	1/15
48	—	0.5	24 hrs	4 × 5	29.8 ± 8.0	332	19.2 ± 2.5	2/20
48	—	1.0	24 hrs	4 × 5	28.2 ± 9.3	309	19.4 ± 3.9	3/20
48	—	1.5	24 hrs	4 × 5	32.4 ± 4.5	370	16.5 ± 5.8	5/20
48	0.5	—	72 hrs	4 × 5	28.7 ± 8.3	316	18.9 ± 3.3	1/20
48	1.0	—	72 hrs	3 × 5	24.7 ± 6.9	259	15.9 ± 3.3	1/15
48	—	0.5	72 hrs	4 × 5	27.8 ± 7.0	303	19.8 ± 2.3	1/20
48	—	1.0	72 hrs	4 × 5	28.0 ± 6.8	306	19.3 ± 1.5	1/20
48	—	1.5	72 hrs	4 × 5	28.1 ± 8.3	307	18.9 ± 3.4	0/20
48	0.5	—	24 and 72 hrs§§	4 × 5	22.8 ± 11.9	230	16.3 ± 5.3	4/20
48	1.0	—	24 and 72 hrs	3 × 5	18.6 ± 9.0	169	14.8 ± 2.8	1/15
48	—	0.5	24 and 72 hrs	4 × 5	29.3 ± 5.2	324	18.1 ± 5.2	7/20
48	—	1.0	24 and 72 hrs	4 × 5	23.8 ± 10.4	244	15.8 ± 2.6	0/20
48	—	1.5	24 and 72 hrs	4 × 5	18.2 ± 6.5	163	15.2 ± 1.7	1/20

*All mice received ip injections of 10^6 L1210 leukemia cells on Day 0. Therapy was initiated on Day 1.

†Given ip on Days 1, 5, 9, 13, and 17.

‡No. of experiments × No. of mice/experiment.

§Mean survival time ± SD (90-day survivors not included in the mean).

¶Mean weight of animals ± SD at death (initial weight at time of inoculation of L1210 cells: 20.0 ± 0.5 g). Weights of 90-day survivors are not included in the mean.

**No. of animals surviving 90 days after cessation of therapy/No. treated. Mean weight at 90 days after therapy: 23.4 ± 2.4 g.

††Dashes = no drug administered.

‡‡Given ip on Day 1 and every 4 days thereafter until death or a total of 5 injections.

§§1 dose ip at each time.

from those obtained with comparable levels of vincristine. Although the weight loss indicated that 1.5 mg/kg of vindesine produced borderline toxicity, its use resulted in the greatest increase in lifespan in the series and produced 25% long-term survivors, a survival rate significantly greater ($P < 0.05$) than that obtained with either concentration of vincristine.

Except in the case of 0.5 mg/kg of vincristine, delay of administration of either vinca alkaloid for 72 hours after methotrexate reduced the increase in lifespan and the number of long-term survivors relative to those seen with a 24-hour delay. The higher level of vincristine was more toxic than when given 24 hours after methotrexate, while the results with vindesine were equivalent to those obtained with vincristine. Administration of two sequential doses of either vinca alkaloid beginning 24 hours after methotrexate and separated by 48 hours produced variable results. At a dose of 0.5 mg/kg, both vinca alkaloids were very effective. Although toxicity reduced the increase in lifespan to an additive level, the sequential doses of 0.5 mg/kg of vincristine produced 20% long-term survivors. The sequential doses of 0.5 mg/kg of vindesine were less toxic than vincristine, producing an increase in lifespan comparable to that seen with a single delayed dose given at 24 hours. More importantly, the sequential doses of 0.5 mg/kg of vindesine produced 35% long-term survivors, a result superior to that achieved with either a single equivalent dose of vindesine given at 24 hours ($P < 0.05$), or with the

TABLE 2.—Treatment of L1210 leukemia* with 72 mg/kg of methotrexate (MTX) ip and vincristine (VCR) ip or vindesine (VDS) ip

MTX† (mg/kg)	VCR (mg/kg)	VDS (mg/kg)	Interval between MTX and vinca alkaloid therapy	Total No. of mice‡	MST ± SD§ (days)	Increase in lifespan (%)	Final weight ± SD¶ (g)	90-day survivors**
—††	—	—	—	4 × 5	6.9 ± 0.6	—	21.3 ± 2.0	0/20
—	0.5‡‡	—	—	3 × 5	9.3 ± 2.2	34	18.4 ± 2.9	0/15
—	1.0‡‡	—	—	2 × 5	12.3 ± 2.4	78	17.6 ± 0.9	0/10
—	—	0.5‡‡	—	3 × 5	8.7 ± 0.6	26	21.3 ± 1.4	0/15
—	—	1.0‡‡	—	3 × 5	10.5 ± 2.6	53	20.5 ± 1.8	0/15
—	—	1.5‡‡	—	3 × 5	12.9 ± 2.1	87	19.3 ± 2.1	0/15
72	—	—	—	4 × 5	22.5 ± 2.0	226	19.9 ± 2.1	0/20
72	0.5	—	Together	3 × 5	24.2 ± 9.8	251	17.8 ± 3.9	0/15
72	1.0	—	Together	2 × 5	17.3 ± 6.3	150	13.5 ± 3.7	0/10
72	—	0.5	Together	3 × 5	26.0 ± 7.4	277	19.4 ± 3.1	0/15
72	—	1.0	Together	3 × 5	29.4 ± 4.8	326	19.4 ± 2.8	0/15
72	—	1.5	Together	3 × 5	31.6 ± 10.3	358	18.8 ± 4.2	0/15
72	0.5	—	24 hrs	3 × 5	24.0 ± 11.2	248	16.8 ± 3.8	3/15
72	1.0	—	24 hrs	2 × 5	18.6 ± 8.4	169	13.3 ± 3.2	1/10
72	—	0.5	24 hrs	3 × 5	36.5 ± 11.8	429	18.0 ± 3.2	9/15
72	—	1.0	24 hrs	3 × 5	33.7 ± 10.3	389	20.0 ± 3.2	4/15
72	—	1.5	24 hrs	4 × 5	30.1 ± 6.5	336	19.6 ± 1.5	4/15
72	0.5	—	72 hrs	3 × 5	26.7 ± 8.3	286	15.5 ± 5.2	0/15
72	1.0	—	72 hrs	2 × 5	24.5 ± 13.0	255	16.4 ± 5.3	0/10
72	—	0.5	72 hrs	3 × 5	25.5 ± 11.2	269	18.2 ± 3.9	2/15
72	—	1.0	72 hrs	3 × 5	18.5 ± 5.0	167	14.0 ± 2.3	2/15
72	—	1.5	72 hrs	3 × 5	21.2 ± 12.4	208	16.6 ± 2.9	0/15
72	0.5	—	24 and 72 hrs§§	3 × 5	16.7 ± 7.5	142	14.5 ± 2.8	1/15
72	1.0	—	24 and 72 hrs	2 × 5	12.5 ± 2.0	81	13.0 ± 1.7	0/10
72	—	0.5	24 and 72 hrs	3 × 5	20.7 ± 9.9	200	15.3 ± 3.4	0/15
72	—	1.0	24 and 72 hrs	3 × 5	25.4 ± 11.8	268	17.5 ± 3.5	2/15
72	—	1.5	24 and 72 hrs	3 × 5	20.4 ± 11.5	195	15.4 ± 3.3	0/15

*All mice received ip injections of 10^6 L1210 leukemia cells on Day 0. Therapy was initiated on Day 1.

†Given ip on Days 1, 5, 9, 13, and 17.

‡No. of experiments × No. of mice/experiment.

§Mean survival time ± SD (90-day survivors not included in the mean).

¶Mean weight of animals ± SD at death (initial weight at time of inoculation of L1210 cells: 20.0 ± 0.5 g). Weights of 90-day survivors are not included in the mean.

**No. of animals surviving 90 days after cessation of therapy/No. treated. Mean weight at 90 days after therapy: 24.1 ± 2.5 g.

††Lashes = no drug administered.

‡‡Given ip on Day 1 and every 4 days thereafter until death or a total of 5 injections.

§§1 dose ip at each time.

sequential doses of 0.5 mg/kg of vincristine (P = 0.05). Although there was an occasional long-term survivor, the weight loss indicated that the higher levels of either vinca alkaloid produced excessive toxicity when administered on this schedule.

Table 2 shows the therapeutic results obtained with 72 mg/kg of methotrexate alone and in combination with the vinca alkaloids. This level of methotrexate was slightly more therapeutic than 48 mg/kg, but it did not produce long-term survivors. The therapeutic effect resulting from the simultaneous administration of methotrexate and 0.5 mg/kg of vincristine was better than that observed with either agent alone, but it represented no more than an additive effect of the two drugs. In contrast, the increase in lifespan following the simultaneous administration of vindesine and the folate analog was greater than an additive effect, but no long-term survivors were produced. Vincristine at a dose of 1.0 mg/kg, when used with 72 mg/kg of methotrexate in either the simultaneous schedule or the delay regimens, was highly toxic. Consequently, two experiments were considered an adequate assessment of the poor therapeutic effectiveness of this level of vincristine.

When vincristine (0.5 mg/kg) or vindesine (0.5–1.5 mg/kg) was given 24 hours after methotrexate rather than with it, further synergism was evident by the increase in lifespan and by the number of long-term survivors. As in experiments with 48 mg/kg of methotrexate (table 1), vindesine produced a therapeutic effect superior to that of vincristine. At equivalent doses of 0.5 mg/kg, vindesine produced 60% long-term survivors as compared to 20% long-

term survivors obtained with vincristine ($P < 0.05$). Higher levels of vindesine produced fewer long-term survivors. It is noteworthy that the therapeutic effect obtained with 72 mg/kg of methotrexate and a 24-hour delay of vinca alkaloid administration was either equivalent or superior to that obtained with any combination schedule employing 48 mg/kg of methotrexate. However, the administration of either vinca alkaloid at 72 or 24 and 72 hours after the higher level of methotrexate was toxic.

These data provide further evidence for a synergistic interaction between methotrexate and vinca alkaloids that is schedule-dependent. Neither the vinca alkaloid nor methotrexate when given alone or simultaneously produces long-term survivors, and, with the exception of vindesine and 72 mg/kg of methotrexate, the increase in lifespan resulting from the simultaneous administration of the two agents is additive. When the vinca alkaloid is given 24 hours after the folate analog, there is an increase in lifespan greater than can be explained by additivity and a significant percent of long-term survivors. There is also a complex relationship between the level of methotrexate and both the time and the number of doses of vinca alkaloid that can be tolerated by the host. The biochemical mechanism responsible for the synergism is unknown. Previous studies (2) from this laboratory have shown that the effect is not related to a vinca alkaloid-mediated alteration in the intracellular level of exchangeable methotrexate. The fact that synergism can be demonstrated when the vinca alkaloid is administered at 72 hours after the folate analog further supports this belief. The enhanced therapeutic effect and the lesser toxicity observed with a 24- versus a 72-hour delay of vinca alkaloid after folate analog suggests that there may be both potentiation of the vinca alkaloid effect and protection from toxicity.

Throughout the study, vindesine appeared more therapeutic than an equivalent dose of vincristine when either was used in combination with methotrexate. Since the mechanism for the synergism is not understood, the reason for the increased effectiveness of vindesine versus vincristine in these mice must await further investigation. Vindesine also has the additional advantage of being less toxic than vincristine, thus permitting a higher level to be administered. Whether the advantage of vindesine over vincristine shown in these animal trials can be demonstrated clinically is unknown at present. However, the flexibility in the time of vinca alkaloid administration plus the ability to utilize either vindesine or vincristine and achieve a positive effect may make this an attractive therapeutic regimen for clinical trial.

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Comparison of the Iv and Ip Routes of Administration of Cisplatin in Dogs[1,2]

R. Gerald Pretorius,[3] Edmund S. Petrilli,[3,*] Charles Kean,[3] Larry C. Ford,[3] James D. Hoeschele,[4] and Leo D. Lagasse[3]

SUMMARY

Cisplatin at a dose of 3 mg/kg was administered to dogs either iv or ip. Cisplatin concentrations in serum, urine, and tissues were measured with a radioisotope tracer method employing ^{195m}Pt cisplatin. Systemic toxicity was monitored by serial BUN, creatinine, and wbc and platelet counts. The mode of administration did not affect systemic toxicity since the changes in renal and bone marrow functions were identical in the two groups. Serum cisplatin levels following iv administration peaked at 13.5 μg/ml at 5 minutes and were biphasic with rapid initial decline and a prolonged elimination phase. In contrast, levels following ip administration increased rapidly to 1.5 μg/ml at 4 hours and then decreased with the iv levels. The amount of drug recovered in the urine was similar regardless of method of administration, with approximately 50% of the injected dose excreted by Day 4. The drug levels within the tissues on Days 4 and 8 were similar, with the exception of the tissues lining the peritoneal cavity. On Day 4 the tissues lining the peritoneal cavity had 2.5–8 times higher levels of drug after ip administration, and this difference was statistically significant ($P < 0.01$). Local toxic effects encountered with ip administration consisted of bloody ascites on Day 4 (four of nine dogs) and filmy adhesions on Day 8 (one of four dogs). It is concluded that ip cisplatin chemotherapy may increase the therapeutic index for small tumors which are confined to the peritoneal cavity.

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Cisplatin, a coordination complex of divalent platinum, has been shown to have chemotherapeutic activity in a wide range of animal and human malignancies. It is active against ovarian carcinoma although its use is often limited by renal, gastrointestinal, and neurologic toxicity (1–8).

Recent studies suggest that the ip route of administration of cytotoxic drugs may enhance the therapeutic index in treating disease limited to the peritoneal cavity. To determine potential differences in therapeutic index related to the method of administration, this report compares the iv and ip routes of administration of cisplatin in dogs, with regard to systemic toxicity, pharmacokinetics, and tissue levels.

MATERIALS AND METHODS

Female mongrel dogs weighing between 10 and 30 kg were used. All dogs had normal pretreatment BUN, creatinine, hemoglobin, wbc and platelet

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[3]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, The Center for the Health Sciences, University of California School of Medicine, Los Angeles.

[4]Oak Ridge National Laboratory, TN.

**Reprint requests to*: Edmond S. Petrilli, MD, Department of Obstetrics and Gynecology, Georgetown University Hospital, 3800 Reservoir Rd, NW, Washington, DC 20007.

counts, bilirubin, and liver enzymes (SGOT and SGPT) and were housed in individual cages for 7 days prior to being included in the study. Before drug administration each dog was anesthetized with pentobarbital iv followed by the placement of a Foley catheter and an internal jugular catheter. All animals were given 1000 ml of Ringer's lactate ip, injected through a 16-gauge catheter over a 10-minute period, and cisplatin at a dose of 3 mg/kg. Dogs treated by the iv route received cisplatin by bolus injection immediately after receiving Ringer's lactate. Dogs treated by the ip route received cisplatin dissolved in Ringer's lactate. Dogs were allowed to recover from the anesthetic and were then given food and water ad libitum. Blood and urine samples were obtained for measurement of cisplatin levels and chemistries (BUN, creatinine, cbc, SGOT, SGPT, and wbc and platelet counts). In the initial phase of the study, an attempt was made to measure all of the above parameters for each animal. Animals were killed at a variety of times from Day 3 to Day 17. After this preliminary work animals were killed on posttreatment Day 4, 8, or 22. Animals killed on Days 4 and 8 were injected with radioactive cisplatin and were utilized for serum, urine, and tissue levels of cisplatin. Due to failure of the Foley catheter and the unwillingness of the laboratory to handle radioactive specimens, not all of the Day 4, Day 8, and preliminary animals had serum chemistries and urine determinations. Animals killed on Day 22 were not injected with radioactive cisplatin and were followed for long-term toxicity and changes in serum chemistries. Cisplatin serum levels for iv and ip administration were constructed from the mean levels of 11 and nine dogs, respectively. The percent of injected dose excreted in the urine was obtained from the mean dose of ten animals in each group. BUN and serum creatinine represent the mean of six or more animals through Day 11 and three animals through Day 22. Wbc and platelet counts are the mean counts of four dogs in the iv group and three dogs in the ip group.

Cisplatin levels in serum, urine, and tissue were measured by a radioisotope tracer method. Cisplatin labeled with ^{195m}Pt, a gamma emitter with a half-life of 4.1 days and a specific activity of 1 millicurie/mg, was provided by the Oak Ridge National Laboratories (TN). ^{195m}Pt-labeled cisplatin (20–50 microcuries) was added to each 3-mg/kg dose of cisplatin. Prior to injection, 5-µl aliquots of the labeled mixture were placed in counting vials containing 1 ml of normal saline. One milliliter of each serum and urine sample and weighed amounts of tissue were counted on a Beckmann gamma counter. By counting the aliquots of the injected mixture at the same time, a ratio of the number of counts per microgram of cisplatin was obtained and the concentration of cisplatin in each of the samples was calculated. A counting error of ± 2 standard deviations on Day 1 was < 2%, while on Day 4, the variation due to counting was 2.5%–5% for an unknown containing 1 µg of cisplatin.

For statistical analysis of the data, we used the biomedical computer program P-series at the Health Sciences Computing Facility, University of California at Los Angeles. Comparison of renal function for each of the two groups was done by a repeated measure analysis. Curve fitting for a two-compartment model was done with a nonlinear, least-squares regression program. Differences in tissue drug levels were computed with a two-tailed Student's t test.

RESULTS

Figure 1 shows the average serum level after an iv bolus injection of 3 mg/kg, with a peak level at 5 minutes of 13.5 µg/ml ± 0.54 (SEM). The distribution phase (α-half-life) was 11.6 minutes and the elimination phase (β-half-life) was 54 hours. After ip administration, serum cisplatin reached peak levels of 1.5 µg/ml at 4 hours, and thereafter levels were similar to the iv levels. Figure 2 shows that the amount of cisplatin excreted in the urine after iv administration was 35% at 4 hours, 40% at 24 hours, and 50% at 4 days. After ip administration, cisplatin excretion in the urine was 10% at 4 hours, 37% at 24 hours, and 46% at 4 days. The excretion on Days 1 and 4 was not statistically different in the two groups ($P > 0.10$).

Cisplatin tissue levels (µg of cisplatin/g of tissue) on Day 4 after treatment are shown in table 1. Tissues with the highest cisplatin concentration were kidney, liver, ovary, uterus, skin, and peritoneum. Tissue levels were similar for ip and iv administration, except for the surface of the peritoneal cavity. Ip administration resulted in higher tissue levels in the diaphragms, mesentery, omentum, and peritoneum. Drug levels within the tissues of the peritoneal cavity were 2.5–8 times higher after ip administration, and with the exception of the peritoneum, all differences were statistically significant ($P < 0.01$).

Tissue levels on Day 8 are shown in table 2. Although the number of animals was too small for statistical evaluation, the differences evident on Day 4 were still present on Day 8. On Day 8, cisplatin levels within the peritoneal surfaces were 3–11 times higher with ip administration.

Drug levels in retroperitoneal lymph nodes were relatively low and did not vary with the route of administration. Ascitic fluid obtained from four of

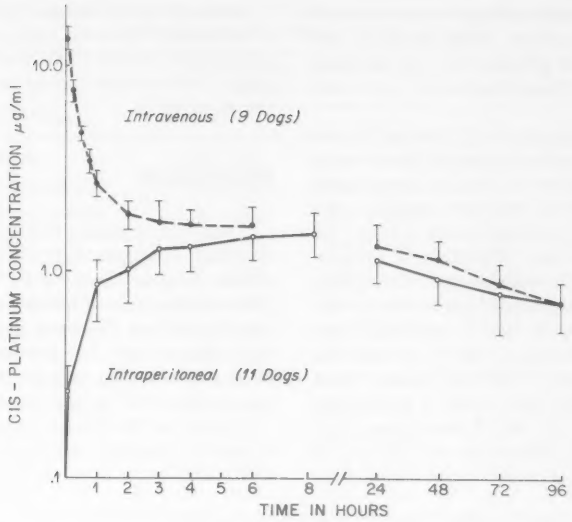

FIGURE 1.—Mean serum cisplatin concentration (µg/ml) + SEM vs time following iv and ip administration of 3 mg/kg of cisplatin in dogs.

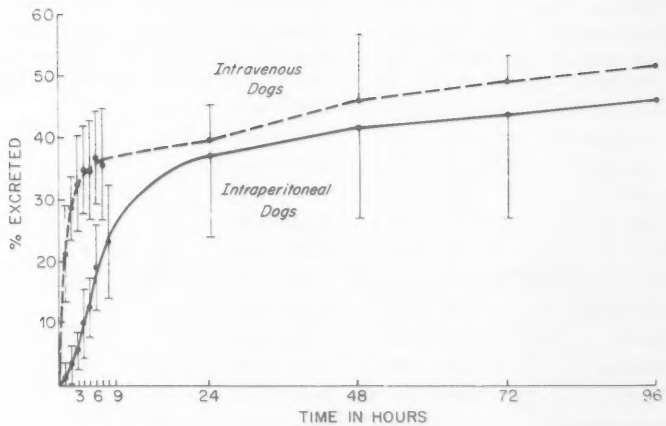

FIGURE 2.—% of injected dose of 3 mg/kg of cisplatin recovered in urine vs time following iv and ip administration in dogs. Each point = average of 10 dogs ± SD.

the ip treated animals on Day 4 had a level slightly lower than that of the serum. Drug levels in one fetus, two amniotic fluid specimens, and multiple cerebrospinal fluid and brain specimens were not detectable.

Toxicity after administration of 3 mg/kg of cisplatin in the dog was pronounced regardless of route of administration. As shown in figures 3 and 4 and as confirmed by repeated measure analysis, the changes in BUN and creatinine were similar for each mode of administration. Hematologic toxicity (figs 5 and 6) was similar for both groups. Most dogs, regardless of group, experienced moderate to severe anorexia and vomiting. SGOT and SGPT remained normal in all animals. Four of 13 animals treated iv showed tubular or interstitial lesions within the kidneys. Four of nine dogs in the ip group had bloody ascites on Day 4. On Day 8 one of four dogs had filmy intraperitoneal adhesions and on Day 22 one of three dogs had a grayish-white membrane covering much of the peritoneal cavity. Microscopic examination revealed that seven of 17 dogs had interstitial or tubular lesions of the kidneys. No pathologic lesions were noted in the bowel in either of the groups.

DISCUSSION

There is increasing evidence to support ip administration of chemotherapeutic agents for intraperitoneal disease. Dedrick et al (9) have published a theoretic analysis of ip drug administration and have concluded that the most useful drugs should have high plasma and low peritoneal clearances. In addition, small tumor size and large fluid volume would be required for ip administration to be effective.

Ozols et al (10) investigated ip and iv doxorubicin in murine ovarian carcinoma; the ip route resulted in 29 long-term survivors out of 40 animals, com-

TABLE 1.—Mean tissue level of cisplatin on posttreatment Day 4 after administration of 3 mg/kg of cisplatin in dogs

Tissue	Mean cisplatin concentration (μg/g ± SEM)		No. of dogs	
	Iv	Ip	Iv	Ip
Serum	0.74 ± 0.08	0.79 ± 0.10	6	7
Kidney cortex	7.47 ± 0.08	7.11 ± 0.97	8	9
Kidney medulla	3.46 ± 0.29	2.48 ± 0.53	4	7
Liver	4.71 ± 0.35	4.92 ± 0.97	8	9
Uterus	4.01 ± 0.48	2.43 ± 1.67	3	2
Skin	4.26 ± 0.99	4.72 ± 3.19	3	4
Ovary	3.04 ± 0.41	2.98 ± 0.37	7	7
Peritoneum	4.03 ± 0.99	11.33 ± 3.34*	4	7
Small bowel mesentery	1.36 ± 0.24	3.86 ± 0.69†	6	7
Right diaphragm	1.30 ± 0.18	7.47 ± 0.81†	7	7
Left diaphragm	1.25 ± 0.19	6.80 ± 1.11†	7	9
Omentum	0.90 ± 0.10	7.46 ± 1.68†	7	9
Spleen	2.03 ± 1.09	2.03 ± 0.78	3	5
Lung	1.96 ± 0.20	1.84 ± 0.28	6	7
Nerve	1.83 ± 0.25	1.57 ± 0.30	4	7
Bile	1.71 ± 0.42	1.84 ± 0.44	6	7
Lymph node	1.69 ± 0.44	2.62 ± 0.94	6	8
Bowel	1.47 ± 0.22	1.27 ± 0.22	7	9
Pancreas	1.25 ± 0.08	1.53 ± 0.26	4	7
Heart	0.68 ± 0.04	0.61 ± 0.10	6	7
Rectus muscle	0.61 ± 0.10	0.91 ± 0.17	7	7
Ascitic fluid		0.52 ± 0.08		4

*$0.055 < P < 0.1$.
† $P \leq 0.01$.

TABLE 2.—Mean tissue level of cisplatin on posttreatment Day 8 after administration of 3 mg/kg of cisplatin in dogs

Tissue	Mean cisplatin concentration (μg/g ± SEM)		No. of dogs	
	Iv	Ip	Iv	Ip
Serum	0.26 ± 0.08	0.43 ± 0.03	3	4
Kidney cortex	6.08 ± 0.26	5.55 ± 0.63	4	4
Kidney medulla	2.70 ± 0.30	2.33 ± 0.27	2	2
Liver	3.18 ± 0.82	4.19 ± 0.40	4	4
Ovary	1.70 ± 0.41	2.82 ± 0.11	3	3
Peritoneum	2.04 ± 0.29	6.90 ± 0.51	2	2
Small bowel mesentery	0.52 ± 0.08	1.71 ± 0.23	2	2
Right diaphragm	0.70 ± 0.10	4.32 ± 0.68	2	2
Left diaphragm	0.64 ± 0.07	4.42 ± 2.14	3	4
Spleen	1.17 ± 0.43	1.17 ± 0.38	2	2
Lung	1.37 ± 0.19	1.25 ± 0.01	2	2
Nerve	1.47 ± 0.21	1.06 ± ——	2	1
Bile	1.29 ± ——	0.49 ± 0.24	1	2
Lymph node	1.34 ± 0.34	1.25 ± 0.06	2	2
Bowel	0.60 ± 0.06	1.14 ± 0.34	3	4
Pancreas	1.00 ± 0.11	0.72 ± 0.16	2	2
Heart	0.42 ± 0.005	0.42 ± 0.02	2	2
Rectus muscle	0.40 ± 0.01	0.47 ± 0.08	2	2

pared to no survivors in a group given the same dose iv. Fluorescence localization showed more doxorubicin in the outer four to six cell layers of solid tumor after ip than after iv administration. Drug levels in ascitic tumor cells after ip injection were 50 times higher than after iv injection. Ip methotrexate (11,12), doxorubicin (13), and 5-FU (14) have been administered to patients; the peritoneal concentration of the drugs was significantly higher than the serum concentration, with a peritoneum to plasma ratio of 900 to 10. Complications associated with repeated ip administration of chemotherapy were abdominal pain and bacterial peritonitis.

In this study, a dose of 3 mg/kg [a relatively high dose in the dog (15)] was chosen because it caused reversible toxicity. One liter of Ringer's lactate was

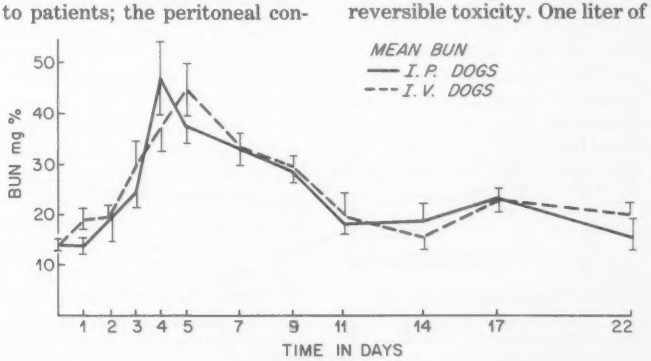

FIGURE 3.—BUN vs time following iv and ip administration of 3 mg/kg of cisplatin in dogs. Points = mean ± SEM of ≥ 6 animals through Day 11 and of 3 animals through Day 22.

given ip to each of the iv treated dogs in an attempt to control hydration-related effects on renal toxicity. The volume of fluid administered ip was extrapolated from data on radioisotope studies in monkeys in which 250 ml injected into a 5-kg animal resulted in uniform distribution of fluid within the peritoneal cavity (16). The radioisotope tracer method of measuring cisplatin has the same major drawback as does the atomic absorption spectroscopy method in that it actually measures platinum concentration rather than cisplatin activity. Equating platinum concentration with cisplatin activity is difficult since it has been shown that cisplatin is metabolized in vivo (17) and that cisplatin is bound to plasma proteins and is apparently inactivated by that binding (18,19). In spite of this, the finding that tissue levels of platinum within peritoneal surfaces are higher following ip administration is significant because it shows a difference in drug distribution.

As has been found in previous studies in dogs (20–23), rats (21,24), and man (17,25), the serum cisplatin concentration following an iv dose decreased in a biphasic manner, with a short initial half-life and a prolonged biologic elimination phase. With ip administration, the initial concentration of cisplatin within the peritoneal cavity varied with the size of

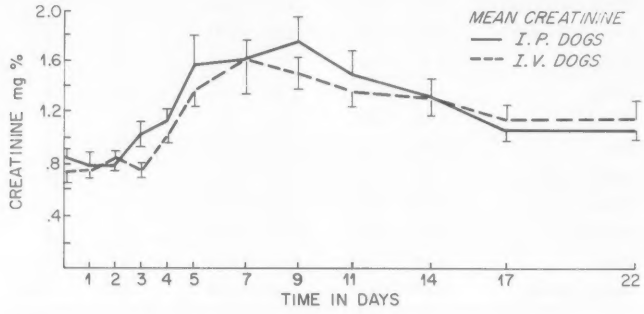

FIGURE 4.—Serum creatinine vs time following iv and ip administration of 3 mg/kg of cisplatin in dogs. Points = mean ± SEM of ≥ 6 animals through Day 11 and of 3 animals through Day 22.

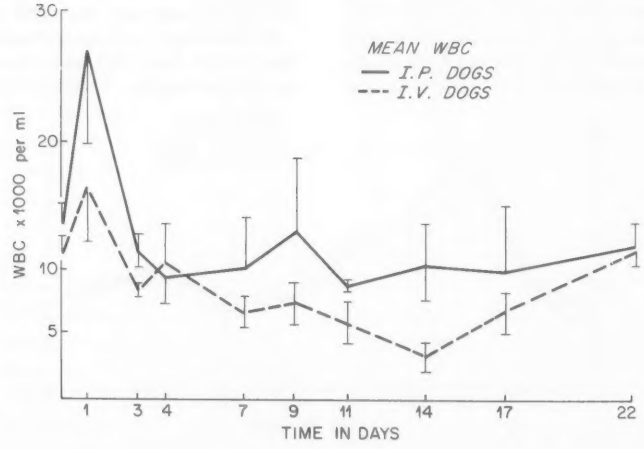

FIGURE 5.—Wbc count vs time following iv and ip administration of 3 mg/kg of cisplatin in dogs. Points = mean ± SEM of 3 dogs in the iv group and 4 dogs in the ip group.

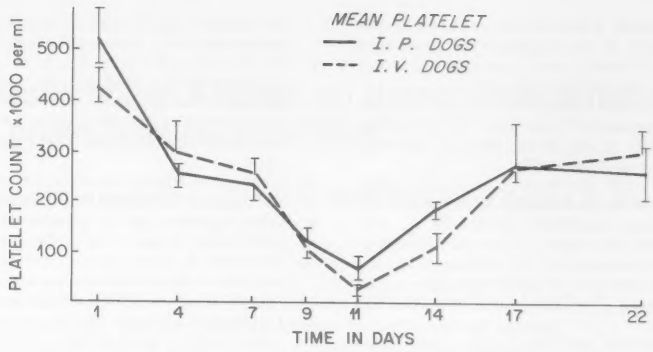

FIGURE 6.—Platelet count vs time following iv and ip administration of 3 mg/kg of cisplatin in dogs. Points = mean ± SEM of 3 dogs in the iv group and 4 dogs in the ip group.

the dog, ranging between 43 and 82 μg/ml. This level exceeds the peak concentration in serum after an iv bolus by a factor of three to six and is similar to the observed increase in tissue levels. The total exposure within the peritoneal cavity may be significantly more than three to six times higher because the serum level decreases at a much faster rate than does the ip level. The serum level following iv bolus has an α-half-life of 11.6 minutes. Jones et al (11) found that 24.5% of an ip injected dose of cisplatin was absorbed within the first hour in rats. This converts to a half-life of 2.4 hours. In man, peritoneal dialysis studies suggest that molecules with low lipid solubility (eg, cisplatin) are absorbed from the peritoneal cavity inversely proportional to the square root of their molecular weight (26,27). Extrapolation of these data to cisplatin with a molecular weight of 300 results in an estimate of the half-life within the peritoneal cavity of 1.3 hours. Neither the data from rats (half-life = 2.45 hours) nor the data from man (half-life = 1.3 hours) can be strictly applied to dogs because the half-life is a function of volume, permeability, and peritoneal surface area, all of which change with changes in experimental design (28). It can be concluded that the half-life after ip administration is on the order of hours rather than minutes. It would then appear that the cisplatin concentration within the peritoneal cavity exceeds the peak iv serum level and decreases at a much slower rate. This longer exposure to higher concentration of drug accounts for the higher tissue levels seen with ip administration.

The finding that at the doses used in this study, systemic toxicity is the same for both iv bolus and ip routes of administration suggests that the higher peak serum levels after iv injection are less important than the similar levels of the elimination phase. Administration of cisplatin by prolonged infusion is also associated with low peak levels and a prolonged elimination phase and might be expected to have similar toxicity. Although local toxic effects following ip administration were not life-threatening, the finding of bloody ascites and filmy adhesions is significant. The relatively high dose of cisplatin administered could be a contributory factor, but further studies in animals and in phase I trials in man would be necessary prior to therapeutic trials of ip cisplatin in man. The finding that increased drug levels can be obtained in the peritoneal tissues suggests that ip cisplatin may increase its therapeutic index.

A slow rate of diffusion of drugs through tissue is a limiting factor of ip drug administration. Ozols et al (10) showed that ip drug penetrated only four to six cell layers in solid tumors. Our own data showing no change in ovarian or small bowel tissue levels also suggest that drug diffusion may be an important factor. Situations where ip administration may be useful include minimal residual disease confined to the peritoneal cavity and positive cytology. With respect to cisplatin, a volume of 2 liters and a dose no higher than currently used iv doses (50–100 mg/m^2) seem appropriate for human investigation. Repeated dialysis with shorter time intervals and lower doses is an alternative.

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Effect of 4′-Doxorubicin Analogs on Heterotransplantation of Human Tumors in Congenitally Athymic Mice[1,2]

Fernando C. Giuliani,[3,4,*] Antonio K. Coirin,[3] M. Rene Rice,[3] and Nathan O. Kaplan[3,5]

SUMMARY

The antitumor activity of three new doxorubicin (DX) derivatives with less cardiotoxicity than the parent compound was tested against several human tumors representative of some of the major classes of human cancer. The tested DX derivatives, modified on the 4′ position of the amino sugar, were 4′-epiDX, 4′-deoxyDX, and 4′-*O*-methylDX. Fourteen human tumors (three breast tumors, three lung tumors, three melanomas, two ovarian tumors, one prostate tumor, one sarcoma, and one larynx tumor) serially transplanted in athymic mice were used to screen the antineoplastic activity of the 4′-DX derivatives. BALB/c nude mice were treated iv with equitoxic doses of each as a single agent (≤ LD10) on a weekly basis for 3–4 weeks, starting when the tumor became relatively large. 4′-EpiDX, which has a higher threshold limit of cardiac toxicity in man, was found active against breast, lung (epidermoid and oat cell carcinoma), prostate, and ovarian tumors. This drug showed particularly good activity against melanomas. 4′-DeoxyDX was active against breast and prostate tumors, while 4′-*O*-methylDX was active against breast and ovarian tumors and possibly sarcoma.

[Cancer Treat Rep 65:1063–1075, 1981]

In order to find new anthracyclines with higher antitumor activity and without dose-limiting cardiotoxicity, considerable attention has been devoted in recent years to investigation of new derivatives of doxorubicin (DX) and daunorubicin (1–7). Of the large number of new derivatives and analogs of DX investigated, compounds bearing modification at the 4′ position of the amino sugar showed interesting properties (8–11).

In this paper we will describe the experimental antitumor activity of three new 4′-DX analogs against several types of human tumors, representative of the major classes of human cancer, heterotransplanted into athymic mice. The DX derivatives under study were 4′-epiDX (epiDX), 4′-deoxyDX (deoDX), and 4′-*O*-methylDX (O-DX) (fig 1).

EpiDX has displayed noticeable antitumor activity against experimental tumors in mice (9,12); toxicity studies in normal and tumor-bearing mice showed that epiDX was less toxic and cardiotoxic than DX (11,12). Even in clinical studies this DX derivative showed lower acute toxicity in phase I trials and was active against some human tumors in early phase II studies (13,14).

DeoDX and O-DX showed minimal cardiotoxicity compared to the parent compound DX in mouse and rabbit systems and demonstrated experimental antileukemic activity (11). Using the athymic mouse-human tumor system, we observed an interesting

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[3]Department of Chemistry, Cancer Center, University of California, San Diego at La Jolla.

[4]On leave from Experimental Division of Oncology B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.

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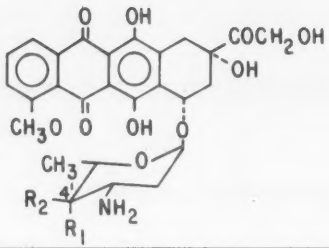
*_Reprint requests to:_ Fernando C. Giuliani, PhD, Experimental Division of Oncology B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milan, Italy.



COMPOUND	R_1	R_2	MOL. WT.*
DX	OH	H	579
4'-epiDX	H	OH	579
4'-deoDX	H	H	563
4'-ODX	OCH_3	H	593

*as hydrochlorides

FIGURE 1.—Chemical structure of DX and 4'-DX derivatives.

antitumor activity of deoDX and O-DX against human colon tumors (15,16). The results described in this paper indicate that the three anthracycline derivatives appear to be good candidates for clinical trials and that the athymic mouse system has great potential for the identification of new anthracycline analogs, or in general, new anticancer agents of clinical interest.

MATERIALS AND METHODS

Animals and Tumors

The animals used in these experiments were 8–12-week-old congenitally athymic BALB/c mice of both sexes, homozygous for the *nu/nu* allele; all were bred in our laboratory. As previously described (16,17), the mice were maintained under conventional conditions in autoclaved cages with polyester fiber filters. Every 3 months, piperazine hexahydrate was added to the drinking water for 3 weeks to eliminate intestinal nematodes such as *Aspicularis tetraptera* and/or *Syphacia obvelata* (18).

Human tumor transplants were established directly by inoculation of fresh tumor tissue from patients into nude mice in our laboratory, as previously described (16). The tumors had been maintained by several passages in vivo. The tumor mass was removed under sterile conditions, separated from the capsule, minced in sterile medium, and then inoculated sc on the backs of nude mice (0.2 ml/mouse) through an 18-gauge needle. The take rate for all

TABLE 1.—Characteristics of human tumor xenografts studied

Tumor No.	Tissue of origin	Histopathologic characteristics	No. of passages*	% mouse LDH activity*
T 112	Breast	Infiltrating and intraductal breast carcinoma	16	20
T 378	Breast	Carcinoma with chondroid change	3	0
T 386	Breast	Infiltrating ductal carcinoma (pseudo medullary type)	6	0
T 222	Lung	Epidermoid carcinoma	21	14
T 291	Lung	Adencarcinoma	11	5
T 293	Sternum	Oat cell carcinoma	18	5
T 242	Lymph node	Malignant melanoma	25	9
T 354†	Melanoma	Amelanotic malignant melanoma	5	7
T 355†	Melanoma	Malignant melanoma (melanotic)	3	10
			4	6
T 17	Ovary	Cystoadenocarcinoma	13	10
T 385	Ovary	Adenocarcinoma	3	0
T 198	Prostate	Adenocarcinoma	13	13
			16	0
			18	0
T 23	Shoulder	Undifferentiated sarcoma	13	14
			15	4
			19	0
T 323	Larynx	Well-differentiated epidermoid carcinoma with parakeratosis	10	10

*At the time of the experiment.
†Established from 2 separate biopsies of a malignant melanoma from the same patient.

the tumor lines was 90%–100%. The tumor donor patients had not received any DX or other antitumor chemotherapy before surgery. At the time of the study, the histologic appearance of serially transplanted tumors was identical to that of the original tumors. Before the transplant, the amount of the mouse LDH was quantified as previously described (16). The maximum allowance of mouse LDH in human tumor tissue was arbitrarily fixed at 20%. The main characteristics of the tumors used are listed in table 1, and their growth curves are shown in figure 2.

TABLE 2.—Experimental chemotherapy for human tumors heterotransplanted into nude mice*

	Amount of drug given per injection (mg/kg)												
	EpiDX				DeoDX		O-DX			DX†			
Tumors	6	8	12	15	4.5	6	4	4.5	6.5	4.4	6	6.6	10
Breast													
T 112		+++‡	+++‡		+++‡	+++‡		+++‡	+++++‡		+++‡		+++‡
T 378				+++‡		++‡			++‡				+++‡
T 386				+++‡		+++‡			+++‡				+++‡
Larynx													
T 323			−	±	−	+	±		+‡		+		−
Lung													
T 222	++‡	+++‡				+	±		+	−		++‡	
T 291			−	−	−	−	−		−		−		−
T 293				+++++‡									++++‡
Melanoma													
T 242			+++	+++	++	++	−		±		+		++‡
T 355§¶		+++++‡	+++++‡									++‡	+++‡
T 355§			±	+‡	−	−	−		−			±	+
T 354				++‡		−			−				+
Ovary													
T 17		−	+			−	−		−		−		±
T 385				++‡	+	+			++‡				+
Prostate													
T 198 (13th passage)		−	+		+++	+++‡					−		+++‡
T 198 (16th passage)				+‡		++‡			+				++‡
T 198 (18th passage)									±				
Sarcoma													
T 23 (13th passage)**		−	++		−††	−††	++		+++††	+		+++‡	
T 23 (15th passage)				++		±			++‡				+++‡
T 23 (19th passage)					−	±			−				

*3 or 4 iv treatments, 1 each week, were started when the tumor became relatively large. Symbols in table = T/C: − = > 50%; ± = ≈ 50%; + = 35%–50%; ++ = 20%–35%; +++ = < 20%; and ++++ = regression to a volume smaller than that at the start of treatment.

†Data from ref 17.

‡Statistically significant as evaluated by Student's t test.

§2 xenografts established from biopsies of separate sites from the same patient.

¶Treatment started 6 days after tumor transplantation.

**4 iv treatments twice a week for 2 weeks.

††Toxic treatment.

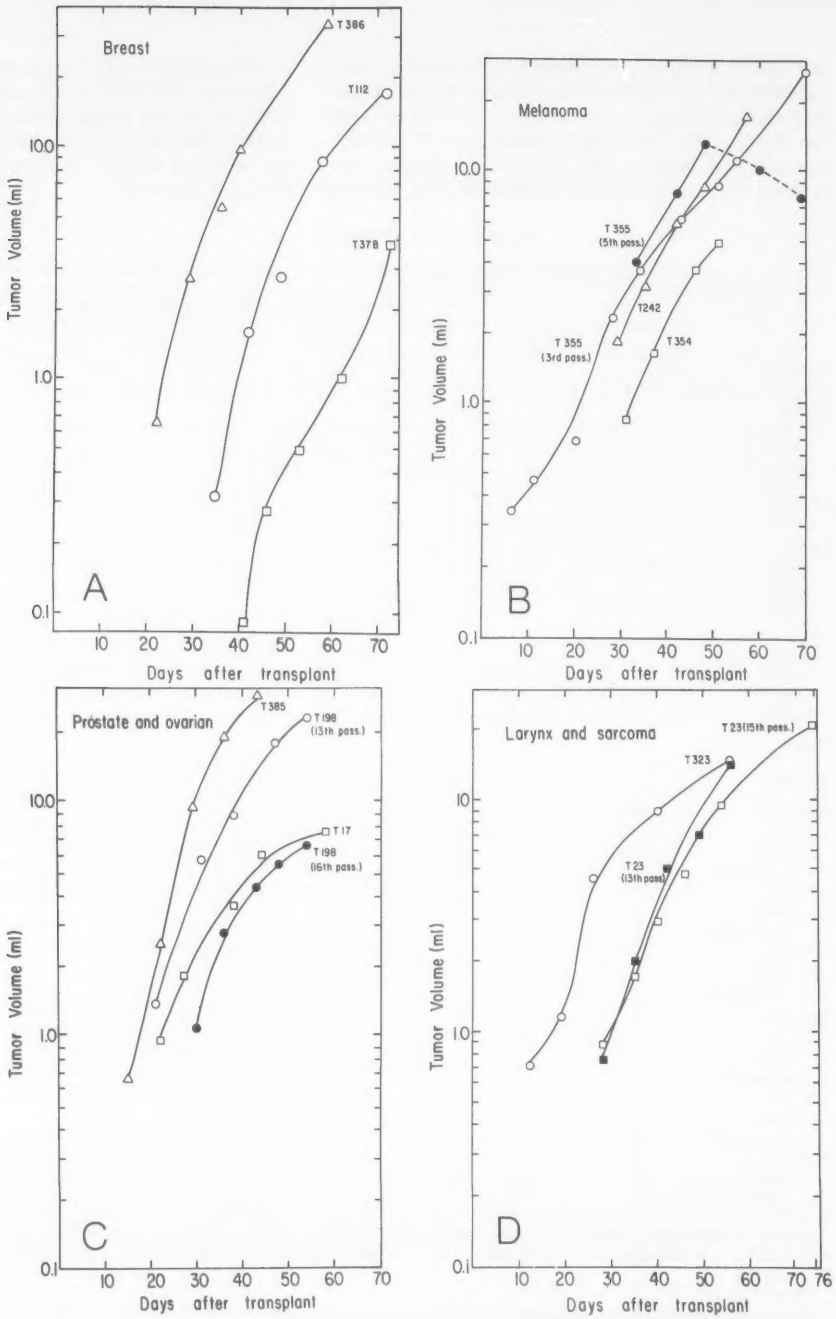

Breast
T 386
T112
T378
Tumor Volume (ml)
100
1.0
0.1
A
10 20 30 40 50 60 70
Days after transplant
Melanoma
T 355 (5th pass.)
T242
T 355 (3rd pass.)
T 354
10.0
1.0
0.1
B
10 20 30 40 50 60 70
Days after transplant
Prostate and ovarian
T385
T 198 (13th pass.)
T 17
T 198 (16th pass.)
100
1.0
0.1
C
10 20 30 40 50 60 70
Days after transplant
Larynx and sarcoma
T 23 (15th pass.)
T 323
T 23 (13th pass.)
10
1.0
0.1
D
10 20 30 40 50 60 70 76
Days after transplant

FIGURE 2.

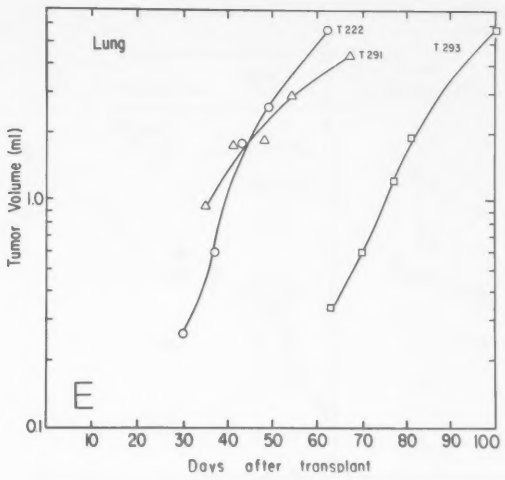


FIGURE 2A–E.—Growth of human tumor xenografts in nude mice. Each point = mean of 6–10 tumors.

Measurement of Tumor Size

The mice were randomized in groups of six to ten animals each when the tumors reached palpable size. The tumors were measured weekly in three dimensions with a slide caliper. The tumor volume was calculated by the equation $\frac{1}{2} \pi d_1 \times d_2 \times d_3 = 1.57 d_1 \times d_2 \times d_3$, where the values of d are the experimental measurements (in millimeters) of length, width, and thickness. Each tumor volume was then expressed in relative tumor volume by the formula RV = Vx/Vi, where Vx is the tumor volume at any given day and Vi is the initial tumor volume at the start of the treatment. Each time the tumors were measured, control relative tumor volumes were compared with treated relative tumor volumes by Student's t test.

Chemotherapy and Evaluation of Chemotherapeutic Effect

EpiDX, deoDX, and O-DX were supplied by Farmitalia Carlo Erba, Milan, Italy. All the compounds have been synthesized by Arcamone and co-workers of the Farmitalia Carlo Erba Research Labs (8,10,12). Drugs were prepared in distilled water at concentrations such that the dose could be given in a volume of 0.1 ml/10 g of body weight and were administered iv on a weekly basis for 3–4 weeks. The three analogs were always tested in one assay (except for the T 222 tumor).

Since individual growth of implanted tumors varies greatly, working with tumors of uniform size was impossible in the large number of mice used in our experiments. Therefore, the treatment was started when transplanted tumors became 0.6–1.5 ml in size. The doses were selected on the basis of toxicity studies performed in our laboratory on non-tumor-bearing BALB/c nude mice; the treatments were performed with equitoxic doses of the single agents (≤ LD10). The LD10 values for the 4′-DX derivatives are (as a chronic toxicity): epiDX, 18 mg/kg; deoDX, 7 mg/kg; and O-DX, 7.5 mg/kg. The relative tumor volume of the treated group (T) was calculated as a percent of that of the control group (C) each time the tumors were measured; the lowest value was expressed as an optimal T/C (%) for each group.

RESULTS

Table 2 summarizes the antitumor activity of the 4′-DX derivatives against seven different types of human tumors transplanted into nude mice.

Breast Tumors

The growth curves of the T 112, T 378, and T 386 breast tumors are shown in figure 3. The 4′-DX derivatives were all active against the human breast tumors tested (table 3). EpiDX administered iv three times, once each week, caused a statistically significant tumor growth inhibition of the three breast tumors tested. Even the treatment with deoDX and O-DX delayed the tumor growth to such an extent that it produced a statistically significant reduction. In the case of the T 112 tumor, O-DX at the higher dose tested (6.5 mg/kg/injection) gave a transient regression between the second and third treatments as well.

Larynx Tumor

Against the T 323 tumor, epiDX and deoDX administered three times iv were not very active at any dose tested (table 3). O-DX at a dose of 6.5 mg/kg/injection gave a statistically significant reduction of the tumor volume.

Lung Tumors

Table 4 and figure 4 describe the activity of DX derivatives against three histologically different lung tumors. The activity of DX derivatives against T 222 was tested in two different experiments. The three iv treatments with deoDX or O-DX at the

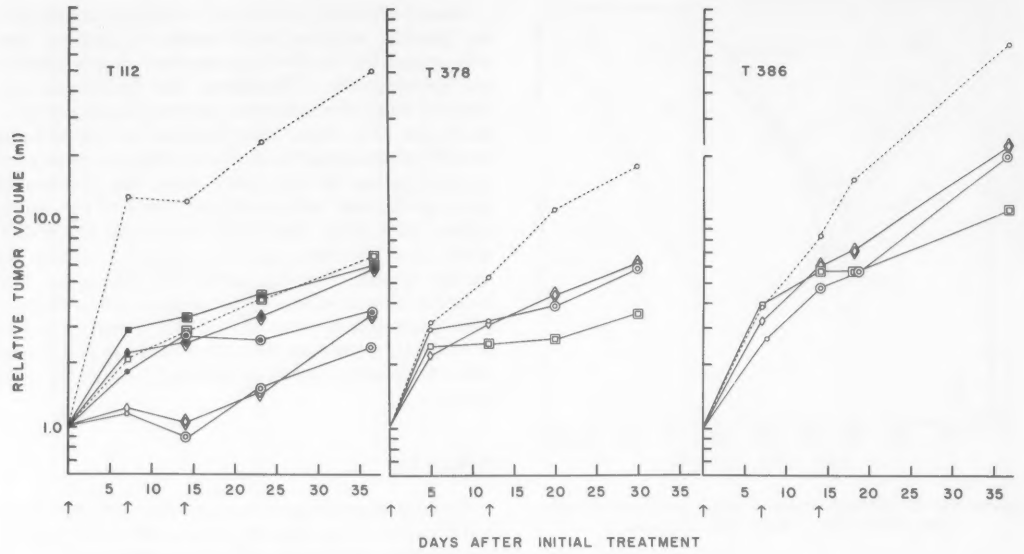


FIGURE 3.—Response of T 112, T 378, and T 386 human breast tumors in mice to 4′-DX derivatives. ○- - -○ = controls; □——□ = epiDX (15 mg/kg/injection); ■ = epiDX (12 mg/kg/injection); □- - -□ = epiDX (8 mg/kg/injection); ◇ = deoDX (6 mg/kg/injection); ◆ = deoDX (4.5 mg/kg/injection); ○——○ = O-DX (6.5 mg/kg/injection); ● = O-DX (4.5 mg/kg/injection). Enclosed points = statistically significant points. Relative tumor volume is the ratio of the mean tumor volume of a group at any given time to the mean tumor volume of that group at the start of treatment. Arrow = treatment.

doses tested showed slight activity against T 222 epidermoid carcinoma; no activity was detected against T 291 adenocarcinoma.

In contrast, epiDX caused a statistically significant delay in tumor growth when tested against T 222 and a statistically significant regression of T 293 oat cell carcinomas. However, when tested against T 291 tumor, only a slight delay in tumor growth was detected in the group treated with 15 mg/kg/injection.

Melanomas

The response of different human melanomas to 4′-DX derivatives is shown in table 4 and figure 5. Against metastatic melanoma T 242, three iv treatments with epiDX or deoDX delayed the tumor growth, but not enough to produce a statistically significant reduction. O-DX did not show activity against T 242 tumor.

The iv treatments of T 354 and T 355, using epiDX at a dose of 15 mg/kg/injection, were started when the tumors were quite large (advanced-stage tumor treatment) and produced a delay in tumor growth, with a statistically significant decrease in tumor volume. Melanotic melanoma T 355 was also treated with a different schedule. Treatment was started 6 days after the tumor implantation (early-stage tumor treatment); four iv injections were given with epiDX at doses of 8 and 12 mg/kg/injection. The early-stage tumor was more sensitive than the advanced-stage tumor. Both doses tested gave a statistically significant regression of the tumor.

DeoDX and O-DX did not show any activity on the growth of T 354 and T 355 tumors.

Ovarian Tumors

The experimental activity of 4′-DX derivatives tested against two different ovarian tumors (T 17 and T 385) transplanted into nude mice is shown in table 5 and figure 6. The activity of epiDX against T 17 at a dose of 12 mg/kg/injection administered three times iv was slight and not statistically significant. O-DX and deoDX tested against T 17 tumors in two different passage numbers (13th and 19th, respectively) did not have any effect on tumor development.

In contrast, all three drugs were active against T 385 tumor growth; however, only the activities of epi-DX and O-DX were statistically significant.

TABLE 3.—Effect of DX derivatives against human breast and larynx tumors transplanted into nude mice*

Tumor No.	Drug	Dose (mg/kg/injection)	Time (days)†	Optimal T/C, % ‡	*P* value §
Breast					
T 112	EpiDX	8	34	13.3 (23)	< 0.02
		12		12.2 (23)	< 0.01
	DeoDX	4.5		11.6 (14)	< 0.05
		6		6.4 (14)	< 0.01
	O-DX	4.5		7.1 (23)	< 0.01
		6.5		< 1 ¶(14)	< 0.01
T 378	EpiDX	15	37	19.7 (20)	< 0.005
	DeoDX	6		34.8 (20)	< 0.01
	O-DX	6.5		33.0 (20)	< 0.05
T 386	EpiDX	15	23	16.1 (40)	< 0.01
	DeoDX	6		32.9 (40)	< 0.01
	O-DX	6.5		29.3 (40)	< 0.01
Larynx					
T 323	EpiDX	12	15	73.8 (20)	
		15		50.7 (20)	
	DeoDX	4.5		60.4 (20)	
		6		47.2 (20)	
	O-DX	4		126.0 (26)	
		6.5		42.5 (26)	< 0.05

*3 iv treatments, 1 each week. 6–10 mice were used in each experiment for both control and treated groups.

†No. of days after tumor transplant that the treatment was started.

‡Relative tumor volume in treated group/relative tumor volume in control group × 100 (optimal value). Values in parentheses = day of evaluation after start of treatment.

§Analysis of controls vs treated groups using Student's t test.

¶Tumor regressed to less than the initial volume.

Prostate Tumor

EpiDX, deoDX, and O-DX were tested against T 198 prostate tumor in three different experiments (table 5 and fig 6) involving tumors with different passage numbers (from 13th to 18th). The three iv epiDX treatments delayed tumor growth in both the experiments performed (13th and 16th passages), but only at the 16th passage, at the higher dose tested (15 mg/kg/injection), did epiDX give a statistically significant difference between the treated group and controls. DeoDX was found more active than epiDX against the prostate tumor at the two passages tested (13th and 16th). A slight but not statistically significant activity against tumor growth at the 16th and 18th passages was found after treatment with O-DX at a dose of 6.5 mg/kg/injection.

Sarcoma

The antitumor activity of DX derivatives was tested on three different passages (13th, 15th, and 19th) of the poorly differentiated sarcoma T 23 (table 5). Two different treatment schedules were used. In the first experiment, the drugs were administered twice a week for 2 weeks. EpiDX at doses of 6 and 8 mg/kg/injection did not produce a statistically significant delay in tumor growth. O-DX was active at doses of 4 and 6.5 mg/kg/injection, but with this treatment schedule the drug had a high toxicity. DeoDX was not active.

In the second experiment drugs were administered once a week for 3 weeks. EpiDX tested at a dose of 15 mg/kg/injection against 15th-passage tumor produced a reduction in tumor growth that was not statistically significant. DeoDX at a dose of 6 mg/kg/injection was not active when tested against two different tumor passages (15th and 19th). Against the T 23 sarcoma on the 15th passage, O-DX (6.5 mg/kg/injection) produced a statistically significant delay in tumor growth; however, that activity was not confirmed when we treated the T 23 sarcoma on the 19th passage with the same dose. Of all the human tumors being carried in our laboratory, the sarcoma appears to be the most sensitive to subtle changes.

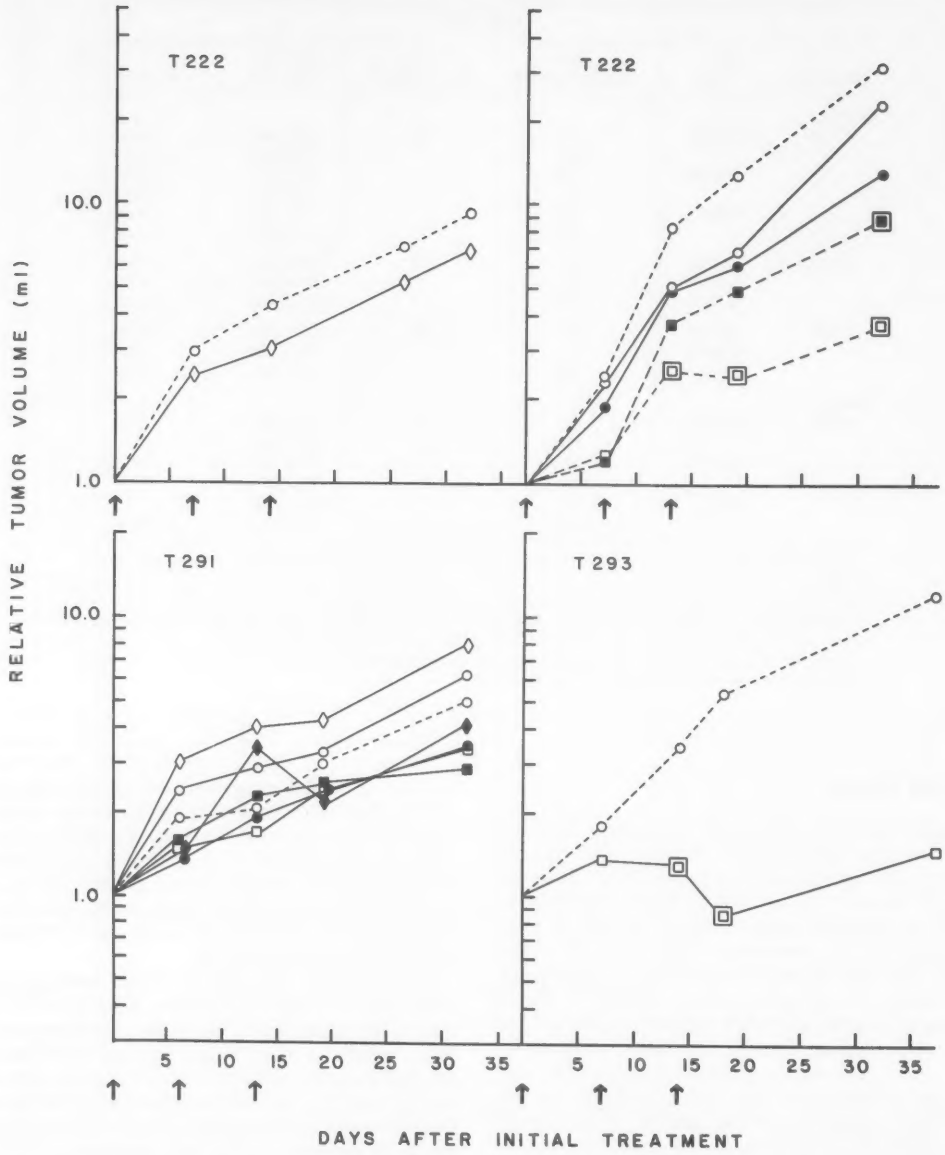


FIGURE 4.—Response of T 222, T 291, and T 293 human lung tumors in nude mice to 4′-DX derivatives. ○- - -○ = controls; ■- - -■ = epiDX (6 mg/kg/injection); □- - -□ = epiDX (8 mg/kg/injection); ■——■ = epiDX (12 mg/kg/injection); □——□ = epiDX (15 mg/kg/injection); ◇ = deoDX (6 mg/kg/injection); ♦ = deoDX (4.5 mg/kg/injection); ○——○ = O-DX (6.5 mg/kg/injection); ● = O-DX (4 mg/kg/injection). Enclosed points = statistically significant points. Arrow = treatment.

TABLE 4.—Antitumor activity of 4′-DX derivatives against human lung and melanoma tumors transplanted into nude mice*

Tumor No.	Drug	Dose (mg/kg/injection)	Time (days)†	Optimal T/C, % ‡	*P* value§
Lung					
T 222	EpiDX	6	30	28.4 (19)	< 0.05
		8		11.8 (19)	< 0.01
	DeoDX	6		69 (14)	
	O-DX	4	21	53.3 (19)	
		6.5		40.9 (19)	
T 291	EpiDX	12	35	68.3 (6)	
		15		57.6 (14)	
	DeoDX	4.5		102 (6)	
		6		74.6 (6)	
	O-DX	4		110.0 (6)	
		6.5		69.3 (6)	
T 293	EpiDX	15	60	< 1 ¶(18)	< 0.01
Melanoma					
T 242	EpiDX	12	29	19.4 (27)	
		15		13.5 (27)	
	DeoDX	4.5		27.5 (27)	
		6		23.5 (27)	
	O-DX	4		88.6 (27)	
		6.5		52.1 (27)	
T 354	EpiDX	15	31	33.0 (20)	< 0.01
	DeoDX	6		77.6 (20)	
	O-DX	6.5		82.5 (20)	
T 355**	EpiDX	8	6	< 1 ¶(22)	< 0.01
		12		< 1 ¶(22)	< 0.01
T 355	EpiDX	12	32	58 (9)	
		15		43 (9)	< 0.01
	DeoDX	4.5		80.5 (9)	
		6		67.9 (9)	
	O-DX	4		64.1 (9)	
		6.5		71.8 (9)	

*Unless specified otherwise, 3 iv treatments were given, 1 each week. 6–10 mice were used in each experiment for both control and treated groups.

†No. of days after tumor transplant that the treatment was started.

‡Relative tumor volume in treated group/relative tumor volume in control group × 100 (optimal value). Values in parentheses = day of evaluation after the start of treatment.

§Analysis of controls vs treated groups using Student's t test.

¶Tumor volume regressed to less than the initial tumor volume.

**The dose schedule used was q7days × 4.

DISCUSSION

In order to improve the therapeutic index of daunorubicin and DX in view of DX cardiotoxicity, a large number of analogs, derivatives, and complexes of the anthracycline antibiotic class have been isolated and synthesized. This study presents data on the experimental antitumor activity of three new analogs modified in the 4′ position of the sugar moiety: epiDX, deoDX, and O-DX. The drugs were tested at equitoxic doses (≤ LD10) established in non-tumor-bearing BALB/c nude mice in our laboratory, as previously suggested by others (19,20). We found that most of the anthracyclines studied were less toxic in athymic mice than in conventional mice (F. C. Giuliani, K. A. Zirvi, and N. O. Kaplan, manuscript in preparation). Houchens et al (21) have reported that nude mice tolerate the same ip dose of DX that is tolerated by conventional mice of the same strain.

It has not yet been clarified to what extent the sensitivity of the xenograft to the antitumor agents corresponds to that of the original patient's tumor. Many authors have reported that the chemosensitivity of xenografts reflects that of the patient's tumors (20,22–24). The general response to chemotherapy of different types of human tumors seems also to resemble that observed in clinical investi-

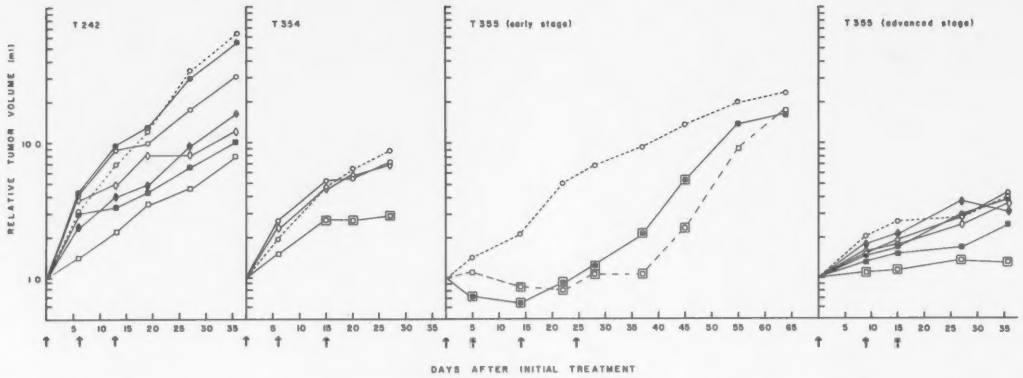


FIGURE 5.—Activity of 4′-DX derivatives against human melanoma tumors in nude mice. *See* figure 4 for definition of symbols.

gations (17,22,25,26). For many reasons, including increased mitotic activity (27), histologic variability (28), and altered immunologic properties (29) of human tumors growing in nude mice, the xenografts do not completely represent the real situation between human host and neoplasia. Even with those limitations we believe that the nude mouse system (human tumor xenograft in athymic mouse recipient) can offer a new model in the search for improved chemotherapy of human cancer and to screen the antineoplastic activity of new drugs. In fact, screening of new agents using nude mouse xenografts has recently been introduced into the US National Cancer Institute preclinical screening procedure.

We have chosen to treat the advanced stage of the tumor essentially because treatment too soon after the transplant might cause interference in the tumor take and because the patient usually receives treatment when the tumor is detectable.

The DX stereoisomer epiDX tested against several human tumors included in our panel was active against the three breast carcinomas tested, T 222 lung epidermoid carcinoma, and T 293 oat cell carcinoma. In addition, antitumor activity was detected against prostate and ovarian tumors and against melanomas. The antitumor activity of epiDX was superior to that of DX against melanomas and T 385 ovarian tumors (table 2).

EpiDX is actually under clinical phase I and II studies at the National Cancer Institute of Milan, Italy (13,14), and at Memorial Sloan-Kettering Cancer Center, New York, NY.[6] The data regarding general toxicity indicated that epiDX showed less acute toxicity than DX although the pattern of side effects was similar to that of the parent compound (13,14). The cardiac toxicity studies indicated that although a significant difference was not observed between epiDX and DX, there was a higher threshold limit for epiDX cardiac toxicity (14). The therapeutic results indicated that epiDX may have a broader spectrum of action with positive effects on melanomas (11,16). This agrees with our experimental data.

DeoDX and O-DX were active against all the breast cancers tested. However, against T 378 breast carcinoma, the antitumor activity of the 4′-DX derivatives was slightly less than that of the parent compound (table 2). DeoDX was also active against two different passages of the T 198 prostate tumor, while human ovarian tumors and sarcomas were sensitive to O-DX. We did not emphasize the activity of O-DX against larynx tumors in this discussion because we want to avoid generalizing and drawing conclusions prematurely from experimentation with one of the few human tumors of the same histologic type. Human tumors of the same type vary widely in their response to anticancer drugs (16,22,30). Consequently, we are planning to increase the number of tumors representing the major classes of human neoplasms in stock in our facility (presently 39). DeoDX and O-DX have not yet been used in clinical trial but are presently under experimental study. In a comparative study of DX, deoDX, and O-DX carried out in mice using the quantitative cardiotoxic test developed by Bertazzoli et al (31), the derivatives were found minimally cardiotoxic (11). Recently, a good antitumor activity of deoDX and O-DX against human colon tumors xenografted in athymic mice has been reported by Giuliani et al (15,16).

[6]Young C. 4′-Epiadriamycin. Recent developments in anthracycline chemotherapy. Presented at New York University Medical Center. June 17, 1980, NY.

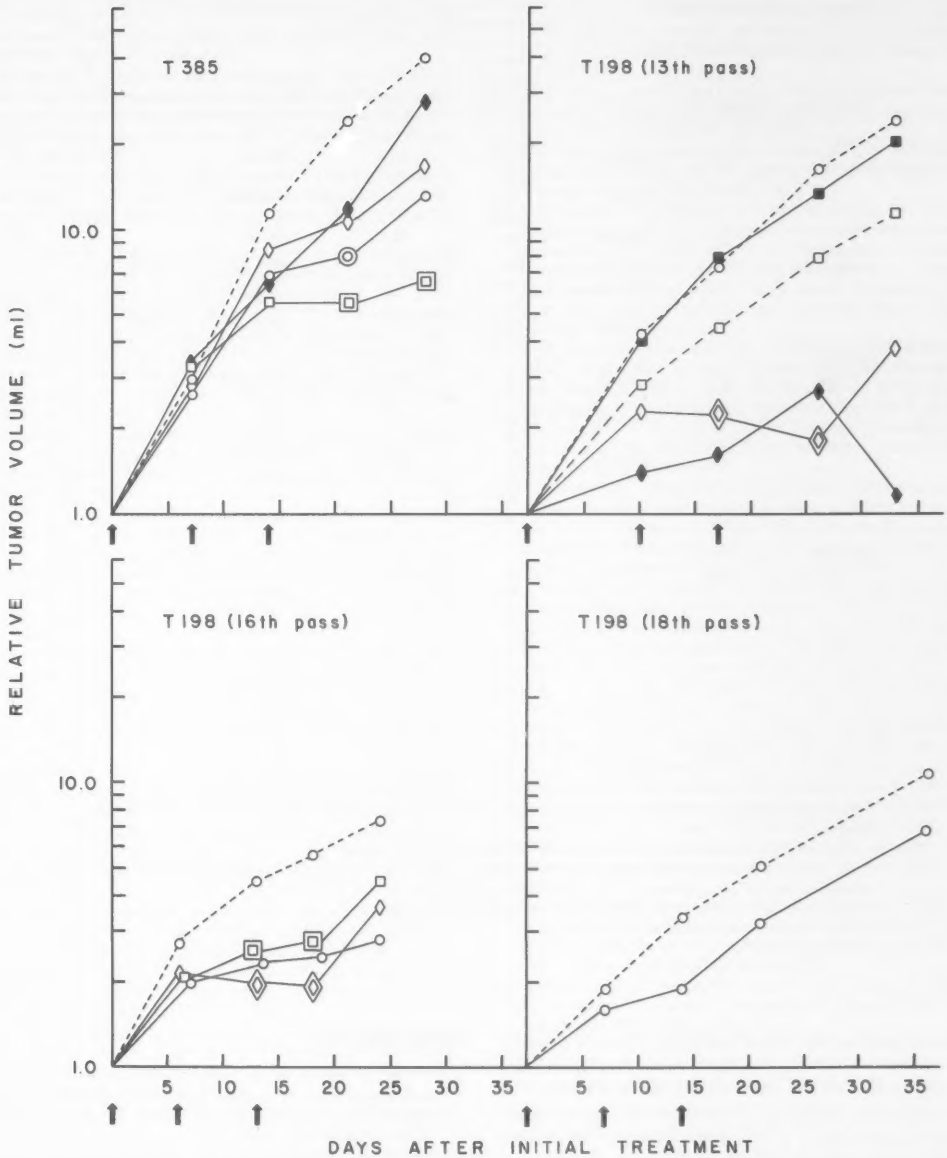


FIGURE 6.—Activity of 4′-DX derivatives against human ovary and prostate tumors in nude mice. *See* figure 4 for definition of symbols.

TABLE 5.—Antitumor activity of 4′-DX derivatives against human ovary and prostate tumors and sarcomas transplanted into nude mice*

Tumor No.	Passage No.	Drug	Dose (mg/kg/injection)	Time (days)†	Optimal T/C,%‡	*P* value§
Ovary						
T 17	13	EpiDX	8	25	102 (34)	
			12		38.6	
		O-DX	4.0		80.6 (34)	
			6.5		63	
	19	DeoDX	6	22	62 (36)	
T 385	3	EpiDX	15	17	23.2 (30)	< 0.05
		DeoDX	4.5		48.6	
			6		44.5	
		O-DX	6.5		33.8	< 0.02
Prostate						
T 198	13	EpiDX	8	21	81.5 (26)	
			12		47.0	
		DeoDX	4.5		11	
			6		9.7	< 0.01
	16	EpiDX	15	30	40.1 (18)	< 0.01
		DeoDX	6		34.8	< 0.01
		O-DX	6.5		43.4	
	18	O-DX	6.5	25	55 (14)	
Sarcoma						
T 23	13¶	EpiDX	6	27	61.9 (18)	
			8		32.6	
		DeoDX	4.5		80**(18)	
			6		78**	
		O-DX	4		26	
			6.5		16**	
	15	EpiDX	15	28	23.5 (20)	
		DeoDX	6		57.1	
		O-DX	6.5	28	35.4 (19)	< 0.05
	19	DeoDX	6		54	
		O-DX	6.5		76	

*3 iv treatments, 1 each week. 6–10 mice were used in each experiment for both control and treated groups.

†No. of days after tumor transplant that the treatment was started.

‡Relative tumor volume in treated group/relative tumor volume in control group × 100 (optimal value). Values in parentheses = day of evaluation after the start of treatment.

§Analysis of controls vs treated groups using Student's t test.

¶The dose schedule used was q3days × 4.

**Toxic dose.

In conclusion, the new DX derivatives are of particular interest because of their lowered cardiotoxicity (especially deoDX and O-DX) and their relatively good antitumor activity. Experimental and clinical results suggest that epiDX has a broader spectrum of antitumor activity, while O-DX and deoDX, in addition to their important anti-colon tumor activity previously reported, possess interesting activity against breast tumors, ovarian tumors, and sarcomas. If the antitumor activity and toxicity of these new compounds are confirmed in clinical trial, we will have achieved a real advancement in human cancer chemotherapy.

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Immunosuppressive Properties and Circulating Life of *Achromobacter* Glutaminase-Asparaginase Covalently Attached to Polyethylene Glycol in Man[1,2]

Abraham Abuchowski,[3] Frank F. Davis,[3] and Stephen Davis[4,*]

SUMMARY

The immunosuppressive effects and circulating life of *Achromobacter* glutaminase-asparaginase (GA) covalently attached to polyethylene glycol (PEG) were examined in human subjects following a single iv dose of 1000 IU/m². Plasma half-life of PEG-GA was 72 hours. Skin test reactivity to recall antigens (mumps and tuberculin) was lost in all four patients tested. In vitro phytohemagglutinin-induced blastogenesis, "natural killing," and phytohemagglutinin-induced cell cytotoxicity was diminished as long as enzyme levels were detectable. In vivo and in vitro activities returned to normal following total plasma clearance of enzyme.

[Cancer Treat Rep 65:1077–1081, 1981]

Enzymes containing glutaminase-asparaginase (GA) activity have been highly successful in inducing remissions in acute lymphoblastic leukemia (1). Major limitations to use of this form of enzyme therapy have been the immunogenicity of the enzymes (2) and the short plasma half-life necessitating constant infusions or frequent injections to deplete plasma glutamine and asparagine.

Modification of the free amino groups on the enzymes has been shown to increase the plasma half-life. Holcenberg et al (3) have treated *Acinetobacter* GA with succinic anhydride. The subsequent succinylated GA preparation produces an enzyme with a plasma half-life of 27 hours in man (3). Uren and Ragin (2) modified *Escherichia coli* and *Erwinia carotovora* GA with a DL-alanine-*N*-carboxyanhydride polymerization technique. The resulting preparation had a plasma half-life of 24 hours in rodents. We have recently modified an *Achromobacter* GA by covalent attachment to polyethylene glycol (PEG). This process renders the enzyme nonimmunogenic as determined by nonproduction of antibodies against the native or modified enzymes or by unaltered blood plasma half-life following repetitive injections in animals (4). If similar data could be demonstrated for man, a new dimension in cancer therapy could be explored.

The immunosuppressive effects of unmodified *Escherichia coli* GA in man are well-known (5). Those effects have been attributed to the glutaminase activity in the enzyme preparations (6). Since immunosuppression depends on the depression of plasma incorporation of amino acids into antigen-responsive lymphocytes (5), the time of administration, dose, and plasma half-life of GA appear to be important. This paper reports the effect of PEG attachment on the plasma clearance of *Achromobacter* GA and the kinetics of its immunosuppressive effects.

PATIENTS, MATERIALS, AND METHODS

Enzyme

Achromobacter GA was obtained from the Centre for Applied Microbiology and Research, Porton,

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[2]Supported in part by Public Health Service grant GM-20946 from the National Institute of General Medical Sciences, National Institutes of Health, Department of Health and Human Services.

[3]Department of Biochemistry and Bureau of Biological Research, Rutgers University, New Brunswick, NJ.

[4]Oncology-Hematology Section, Veterans Administration Medical Center, East Orange, NJ, and the CMDNJ-New Jersey Medical School, Newark.

**Reprint requests to:* Stephen Davis, MD, Oncology/Hematology, Veterans Administration Medical Center, East Orange, NJ 07019.

Salisbury, England. PEG-GA was prepared, as previously described (4). The enzyme was dissolved in 0.1 M borate buffer, pH 9.25. Activated PEG was added with stirring, and the solution was left for 2 hours at 18°C. The solution was dialyzed against phosphate-buffered saline (pH 7.3) in an Amicon thin-channel device equipped with a PM-30 membrane, sterilized by passage through a 0.22-μ Millipore filter, and stored at 4°C. The L-asparaginase activity was 12% of the unmodified enzyme (4). PEG was coupled to 71% of the amino groups (4). L-Asparaginase activity was measured by Steinbeck's modification (7) of the assay system of Jayaram et al as previously described (4).

Preparation of Lymphocytes

Blood was drawn at the designated times by phlebotomy into heparinized syringes. Mononuclear cells were separated on a Ficoll-Hypaque gradient. Morphologically, > 93% of the cells were lymphocytes.

Patients

Four patients with bronchogenic carcinoma were selected for study only on the basis of previously documented tuberculin reactivity. They were male, were 54–69 years old, had not received chemotherapy or radiotherapy within 3 months of testing, and had no significant hepatic or renal dysfunction. Normal controls were healthy hospital personnel. All patients signed informed consents. This protocol was approved by the Human Studies Committee of the Veterans Administration Medical Center.

PEG-GA (1000 IU/m^2) was injected iv over a 30-minute period. Plasma was obtained at the designated time intervals for enzyme activity determination, amino acid analysis, and immunologic studies.

In Vitro Immunologic Assays

Phytohemagglutinin (PHA)-induced cytotoxicity (LICC) was achieved using EL-4 target cells labeled with ^{51}Cr ($Na_2{}^{51}CrO_4$; New England Nuclear Corp, Boston, MA). The EL-4 cell is from a C57BL/6 murine lymphoma maintained in tissue culture (8). EL-4 cells (50 μl; 2 × 10^5/ml) were suspended in medium containing 10% patient serum or normal human AB serum with PHA-M (50 μl; 25 μg/ml) in microtiter wells. Lymphocytes (100 μl) were added at an effector:target cell (E:T) ratio of 20:1. In our laboratory, this ratio yields optimal cytotoxicity. The microtiter plates were centrifuged at 100 × *g* for 3 minutes and incubated at 37°C for 4 hours. Supernatant was removed for counting in a gamma counter. Control wells received 100 μl of RPMI-1640 medium (spontaneous counts per minute [cpm]) or 0.1% NP-40 medium (Shell Oil Co; maximum cpm). Cytotoxic activity was calculated from the following formula:

$$\% \text{ cytotoxicity} = \frac{\text{cpm (experimental)} - \text{cpm (spontaneous)}}{\text{cpm (maximum)} - \text{cpm (spontaneous)}} \times 100.$$

The "natural killing" (NK) assay was performed with K562 target cells. This is a leukemic tissue culture line (9). The cells were prepared and suspended as described above at a target cell concentration of 1 × 10^4. The effector:target cell ratio was 40:1. This ratio yields the optimal NK in our laboratory. The microtiter plates were incubated for 18–24 hours. Each assay was performed in triplicate.

PHA transformation was performed as follows. A 2 × 10^6/ml concentration of mononuclear cells was made by suspending cells in RPMI-1640 medium containing 20% patient serum or normal human AB serum; 100 μl of suspension (2 × 10^5 cells) was placed in microtiter plates with PHA-P to a concentration of 50 μg/ml. Medium without PHA was used as control. Fifty micrograms of ^{125}IUdR and FUdR (0.8 γ/ml) was added to each well at 48 hours of incubation, and incubation continued for 18 hours. Contents of the wells were collected on fiber filters and counted. Results were expressed as a stimulation index (SI):

$$\text{SI} = \frac{\text{cpm (stimulated sample)}}{\text{cpm (sample without mitogen)}}.$$

Student's t test was used for statistical significance.

Lymphocyte Membrane Markers

The percent of lymphocytes with the ability to form rosettes with neuramindase-treated sheep erythrocytes (T cells) and the ability to have positive membrane fluorescence with fluorescein-conjugated polyvalent goat anti-human immunoglobin, $F(ab)'_2$ fragment (B cells) were determined as previously described (10). Normal values are 66.7% ± 9.4% and 9.8% ± 3.2% for T and B cells, respectively.

The normal values stated for all in vitro immunologic parameters were obtained from 62 healthy members of the hospital staff (34–76 years of age).

RESULTS

Plasma Clearance

Figure 1 shows the change in plasma activity of PEG-GA with time following a single injection of

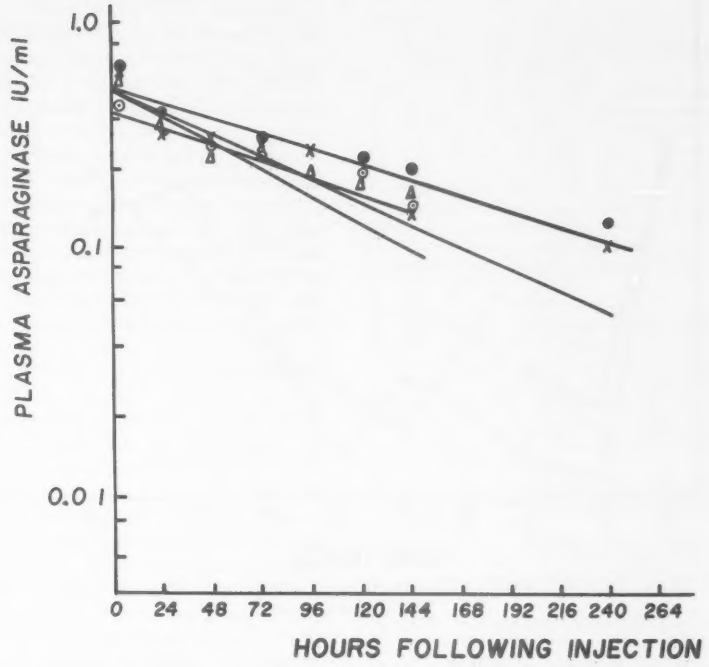

FIGURE 1.—Plasma asparaginase activity after a single dose of 1000 IU/m² of PEG-GA in 4 patients. Symbols = values for individual patients. Lines are drawn through values by log-linear least-squares method.

1000 IU/m². The values shown for the four patients reveal a clearance as a single exponential, ie, there is a linear decrease in activity with time. The plasma half-life was 72 hours. No enzyme activity was detectable after Day 18. The plasma half-life of 12,000 IU of native (unmodified) *Achromobacter* GA was < 30 minutes for the initial phase and 120 minutes for the second phase (data not shown).

Immunologic Studies

The absolute peripheral blood lymphocyte count prior to and during therapy was within normal limits (2100 ± 900/μl) and did not vary significantly during the observation period (1870 ± 490 vs 2020 ± 610, respectively). The ability of lymphocytes to form rosettes with sheep erythrocytes (T cells) and stain for membrane immunoglobulin (B cells) was unimpaired and also remained constant within the normal range. Thus, PEG-GA appears to be nontoxic to normal lymphocytes.

Skin testing to recall purified protein derivative (PPD) and *Candida* antigens was positive (> 10-mm induration and erythema, respectively) in all four patients prior to receiving PEG-GA. Mean induration reaction to PPD was 14.2 mm. When retesting was done on Day 8 following enzyme injection, no delayed hypersensitivity reaction to mumps was detectable. Mean PPD induration reaction was 6.5 mm, with none > 10 mm. A final skin testing was performed on Day 30; the pretest pattern and strength of reactivity returned.

PHA mitotic activity (SI), NK, and LICC were tested before injection of PEG-GA and at designated times thereafter. The mean pretreatment SI for our patients was 62 ± 12.3 (normal: 50–100) and the mean % LICC was 54 ± 8.2 (normal: 45–75). As can be seen in figure 2, both mean SI and mean LICC fell dramatically on Day 3 (SI = 3, range = 1.2–4.1; LICC = 16%, range = 7–31) and remained depressed until Days 22–25 at which time they returned to normal range. At no time did the SI or LICC reach normal immediately following enzyme injection. NK activity in the normal individuals we

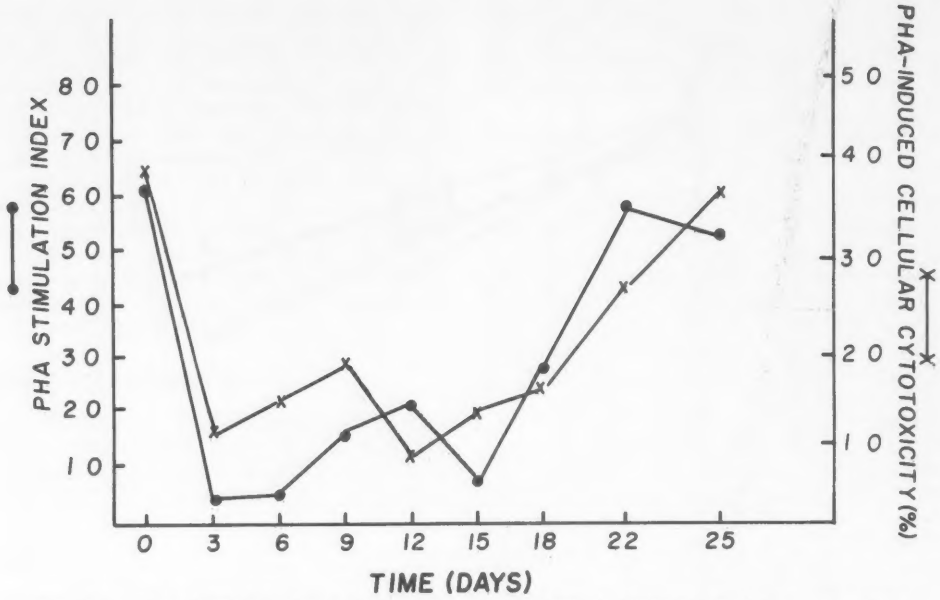

FIGURE 2.—SI and LICC in 4 patients after a single dose of 1000 IU/m² of PEG-GA. Symbols = mean values at specific times indicated.

have tested is 69% cytotoxicity (range: 46%–84%). All four of our patients initially had normal NK activity (table 1). NK markedly decreased in all patients following PEG-GA injection; it did not return to the normal range until Days 22–25.

To assure ourselves that the decreased SI, NK, and LICC were indeed due to depletion of glutamine and possibly asparagine, lymphocytes obtained on Day 3 following PEG-GA were washed free of patient serum, incubated for 24 hours in normal human AB serum, and tested as described. With this procedure, the SI, NK, and LICC were normal for all patients (results not shown).

The *P* value for each day following injection up to Day 18 was ≤ 0.04. However, with only four patients, statistical significance must be interpreted cautiously.

TABLE 1.—NK activity following injection of PEG-GA*

Day	Mean % cytotoxicity	Range
0	62	51–72
3	9	3–29
6	17	7–31
9	7	2–25
12	19	9–30
15	23	13–41
18	11	6–32
22	64	51–75
25	76	48–81

*Normal control values: mean % cytotoxicity ± SE, 69 ± 12; range, 46–84.

DISCUSSION

Our present study demonstrates that the PEG modification of *Achromobacter* GA extends the plasma circulating life of the enzyme. The increase in plasma half-life in man by PEG attachment (72 hours) is superior to the 24-hour half-life obtained by succinylation of *Acinetobacter* GA obtained by Holcenberg et al (3). Our data in humans confirm the data we obtained in rodents (4); our study should be extended to formal phase I studies.

Studies on the immunosuppressive effects of native *Escherichia coli* GA in man have been reported (5). This enzyme was found to inhibit the in vitro PHA blastogenic response as well as the delayed hypersensitivity skin reaction to the primary antigens limpet hemocyanin and the copolymer of glutamic acid, lysine, alanine, and tyrosine (5). The GA did not alter skin reactivity to recall antigens, ie, mumps and dermatophytin. Inhibition of the PHA blastogenic response has been previously demon-

strated in animals (6). The suppression is not due to cytotoxicity but rather to depletion of plasma L-glutamine and L-asparagine (6). In the present study using PEG-GA, we confirm and extend the work of other investigators by showing that PHA blastogenic response is markedly suppressed and that the suppression is probably due to depletion of plasma L-glutamine and perhaps L-asparagine. Similarly, LICC and NK were amino acid-dependent; these recently described cellular functions are thought to be involved in host defenses against infections and neoplasia (9,11,12). It is important to note that none of our patients had any infectious episodes during PEG-GA therapy. The loss of PPD and mumps skin test reactivity was unexpected since Ohno and Hersch (5) failed to demonstrate this phenomenon with doses of *Escherichia coli* GA from 12,000 to 400,000 IU/m²/day for 3–38 days. These investigators suggested that L-asparaginase enters the extravascular spaces with difficulty and may be excluded from sites of delayed reactions or protein synthesis, and that blastogenesis may not be necessary for delayed sensitivity. The fact that delayed sensitivity to recall antigens was lost and then regained in our patients is best explained by the continuous prolonged effects of the PEG-GA.

Similarly, it is important to note that *Escherichia coli* and *Erwinia carotovora* L-asparaginases have 3%–5% glutaminase activity, whereas the *Achromobacter* we used has a greater glutaminase than asparaginase activity. This fact might explain why *Escherichia coli* asparaginase does not diminish recall antigen reactivity.

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Measurement of Urinary β_2-Microglobulin in the Detection of Cisplatin Nephrotoxicity[1]

A. I. Cohen, J. Harberg, and D. L. Citrin[2,*]

Cisplatin is a new anticancer agent with significant activity against a variety of tumors (1). The major toxic effect associated with the drug is renal tubular damage which may cause severe acute renal failure (2). Reduction of acute nephrotoxicity has been achieved by use of pretreatment hydration, mannitol, and furosemide (3,4). Despite these measures, significant reduction in creatinine clearance occurs in many patients after long-term cisplatin therapy. Acute cisplatin nephrotoxicity has conventionally been monitored by serial measurements of serum creatinine, BUN, and creatinine clearance and by detection of proteinuria, but these tests do not predict the development of chronic renal damage.

A potentially more sensitive indicator of cisplatin nephrotoxicity is urinary β_2-microglobulin (β_2M), a low molecular weight protein (11,800 daltons) that is synthesized by most nucleated cells. It is filtered by the glomerular membrane, with nearly complete reabsorption and catabolism in the proximal tubule. Levels in urine are markedly increased following acute tubular injury due to infection (5), heavy metals (6,7), aminoglycoside antibiotics (8), and renal transplant rejection (9). As the major site of cisplatin damage is the renal tubule, we considered that the urinary levels of β_2M might provide a useful clinical index of acute cisplatin nephrotoxicity. We therefore measured β_2M in 35 cancer patients before and 12 hours after cisplatin treatment.

MATERIALS AND METHODS

Thirty-five cancer patients receiving cisplatin were studied. There were 16 males and 19 females (median age, 59 years; range, 18–76). Fourteen patients had breast cancer; seven, lung cancer; seven, bladder cancer; five, prostate cancer; and two, cancer of the testis. Prior to study, all patients had normal serum creatinine (≤ 1.2 mg/dl) and no patient had proteinuria. Patients with active urinary tract infection and patients receiving other potentially nephrotoxic drugs (methotrexate, cephalosporins, or aminoglycosides) were excluded from the study. A single patient with cancer of the bladder and chronic bacteriuria whose prestudy serum creatinine was stable (2.6 mg/dl) was included.

TABLE 1.—Chemotherapy concomitant with cisplatin

Drug	No. of patients
Doxorubicin	30
Cyclophosphamide	22
Hexamethylmelamine	8
Vincristine	5
CCNU	5
Bleomycin	1
None	2

Of the 35 patients studied, 33 received cisplatin as part of combination chemotherapy (table 1). Twenty-five patients had received cisplatin as part of prior chemotherapy (table 2). In all cases, cisplatin was given iv immediately following 2 liters of normal saline with 10 mEq of KCl, which was given over 2 hours. Mannitol was not used routinely, but furosemide (20 mg) was given iv if urine volume decreased to < 150 ml/hour. Chemotherapy was given at approximately 5 PM. Urine collections were made immediately before and 12 hours after cisplatin infusion. The latter specimens, obtained in the early morning, were fresh specimens, collected after the bladder was emptied of overnight urine.

Several control groups were also studied: *drug-treated patients*—12 cancer patients (eight males and four females), median age, 56 years (range, 37–73), who had never received cisplatin and were treated with combination chemotherapy excluding cisplatin and methotrexate; *untreated patients*—11 cancer patients (six males and five females), median age, 60 years (range, 32–79), who had never received cisplatin and had received no other chemotherapy for at least 6 weeks prior to study; and

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[2]Department of Human Oncology and Medicine, University of Wisconsin and Wisconsin Clinical Cancer Center, Madison.

**Reprint requests to:* Dr. Dennis L. Citrin, MD, Division of Oncology, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461.

TABLE 2.—Details of cisplatin dose

Total dose prior to study (mg)	No. of patients	Dose on day of study (mg)	No. of patients
0	10	≤50	7
≤200	10	51–100	25
201–400	6	>100	3
401–800	9		
Total	35		35

healthy subjects—ten healthy subjects (three males and seven females), median age, 53 years (range, 39–67), without a history or clinical evidence of cancer, renal disease, hypertension, or diabetes mellitus.

Initially, 12 cisplatin-treated patients were studied in detail. Accurate 2-hour urine collections were obtained immediately before and 12 hours after a dose of cisplatin. Urine was collected in bags containing 1 M sodium hydroxide titrated to maintain a pH > 6; 0.1 ml of sodium azide was added as a preservative. Serum samples (10 ml) were obtained immediately before and 12 hours after cisplatin therapy. All samples were stored at −20°C until they were analyzed.

The total quantity of β_2M in the 2-hour urine samples was measured using a specific radioimmunoassay (Pharmacia Diagnostic, Inc). All samples were measured in duplicate, and the mean of the two samples was used. Serum levels of β_2M were measured in an identical manner. Pretreatment and posttreatment levels of β_2M in the 2-hour urine specimens and in the sera were then compared by paired Student's t test (two-tailed).

To reduce the need for accurate urine collections, the ratio of β_2M to creatinine in spot urine samples (β_2M/Cr) was measured in an additional 23 cancer patients treated with cisplatin and in the three control groups. Spot urine samples were obtained after double voiding, immediately before chemotherapy, and 12 hours later. In the nontreated cancer patients and healthy subjects, samples were obtained at approximately 6 PM and 6 AM, similar to collection of the pretreatment and posttreatment samples in the drug-treated groups.

Urine samples were collected in plain glass bottles (25 ml) containing 0.4 ml of 1 M sodium hydroxide and 10 mg (0.1 ml) of sodium azide (constituting < 2% of total volume); 25-ml aliquots were also taken from the urine samples of the 12 cisplatin-treated patients initially studied. β_2M levels were measured in all samples, using the method previously described. Urinary creatinine was measured using an automated colorimetric method (Technicon AutoAnalyzer II). All samples were measured in duplicate, and the means of the two samples were used. β_2M/Cr ratios were calculated for the pretreatment and posttreatment urine samples (PM and AM) in all patient groups. Ratios were then compared in each group by the Wilcoxon test for matched samples (two-tailed). Pretreatment β_2M/Cr ratios in each group were compared using one-way analysis of variance.

RESULTS

Thirty-five patients received iv cisplatin (median dose, 85 mg; range, 27–145). Thirty-three patients were given cisplatin in combination with at least one other drug (usually doxorubicin and/or cyclophosphamide) (table 1). Two patients received cisplatin as single-agent therapy. No patient developed clinical or conventional laboratory evidence of acute renal damage. No patient developed proteinuria or an elevated serum creatinine (mean pretreatment serum creatinine, 0.95 ± 0.05 mg/dl; mean posttreatment, 0.94 ± 0.05 mg/dl; $P > 0.2$).

In the 12 patients initially studied with accurate 2-hour urine collections, a significant increase in the total 2-hour β_2M levels was noted 12 hours after cisplatin infusion, compared with the immediate pretreatment level (mean pretreatment, 13.6 ± 2.2 μg/dl; posttreatment, 44.2 ± 14.4 μg/dl; $P < 0.05$, two-tailed Student's t test).

Comparison of urinary β_2M/Cr ratios measured before and 12 hours after treatment with cisplatin in 35 patients also showed a significant increase (table 3). No increase in β_2M/Cr ratios was seen in any control group (table 3).

There was no significant difference between the pretreatment ratio (measured PM) in cisplatin-treated patients and in the three control groups. There was no correlation between the observed change in the β_2M/Cr ratio and previous exposure to cisplatin ($r = 0.46$, $P < 0.1$), nor was there any correlation between the increase in the β_2M/Cr ratio and the study dose of cisplatin ($r_s = -0.04$, $P < 0.2$), using the Spearman rank correlation test.

DISCUSSION

In this study we have demonstrated that 12 hours after treatment with cisplatin there is a significant elevation of urinary β_2M levels (measured as total β_2M and as β_2M/Cr ratios), indicating acute tubular injury. Similar changes were not seen in untreated cancer patients, in cancer patients receiving other chemotherapy, or in healthy controls. The increase in β_2M levels is apparently due to short-term exposure to cisplatin, as prestudy β_2M/Cr ratios were

TABLE 3.—Urinary β_2M/Cr ratios

Group	No. of patients	β_2M/Cr ratio*		P‡
		Pretreatment†	Postreatment	
Cisplatin-treated	35	1.6 ± 0.3	14.1 ± 4.6	< 0.0004
Drug-treated (no cisplatin)	12	0.6 ± 0.2	0.8 ± 0.3	< 0.2
Untreated cancer	11	2.2 ± 1.0	1.7 ± 0.8	< 0.09
Healthy controls	10	1.5 ± 0.6	1.0 ± 0.3	< 0.2

*Values = mean ± SE. β_2M was measured as μg/dl; Cr was measured as mg/dl.
†$P > 0.2$ between groups (one-way analysis of variance).
‡Wilcoxon paired test.

not significantly higher in the patients treated with cisplatin than in the three control groups. This view is further supported by the lack of correlation between the change in β_2M levels and previous exposure to cisplatin.

The duration of the increase in β_2M levels was not addressed in this study, although a previous report by Jones et al (10) suggests that measurable increases in β_2M may be anticipated for at least 7 days following cisplatin treatment. The β_2M/Cr ratios reported in this uncontrolled study (of a small number of patients) were smaller by a factor of ten than those reported here. This probably reflects the fact that in Jones' study, urine was collected without double voiding. β_2M is very labile in an acid pH, and consequently, the urine should either be alkalized in vivo or, as in our study, overnight urine should be discarded, and freshly voided urine should be immediately alkalized prior to freezing and storage.

It is of considerable interest that we have observed significant increases in β_2M despite very modest doses of cisplatin (median dose, 85 mg), which were given with iv fluid and diuretic prophylaxis. These changes reflect subclinical renal damage, as no patient showed conventional laboratory evidence of renal toxic effects (normal posttreatment serum creatinine, and absence of proteinuria). Increases in β_2M levels clearly provide a highly sensitive indicator of renal tubular damage caused by cisplatin. The important clinical question which has not been addressed in this pilot study is whether those patients who demonstrate a sudden elevation of β_2M after cisplatin therapy eventually develop significant subacute or chronic renal damage. A long-term prospective study of serial measurements of β_2M in cisplatin-treated patients is now in progress to precisely define the role of β_2M in the monitoring of renal damage caused by cisplatin.

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Effect of Thymidine on the Toxicity and Antitumor Activity of Ftorafur[1,2,3]

V. Bruce Grossie, Jr,* and Ti Li Loo[4]

SUMMARY

The effects of thymidine (TdR) on the toxicity and antitumor activity of ftorafur (FT), a 5-FU analog, were determined. The LD10 of FT was 130, 430, and 680 mg/kg, respectively, when FT was administered ip in the following treatment schedules: (*a*) daily for 9 days, (*b*) every 4th day for three treatments, and (*c*) 1 day only. When FT was administered simultaneously with 250 mg/kg of TdR, the LD10 was 13, 62, and 630 mg/kg in the respective treatment schedules. Lethargy was observed in mice when the daily dose of FT was ≥ 150 mg/kg. FT alone was active (% treated/control [T/C] = 153) against ascites P388 murine leukemia only at high, single doses. Simultaneous administration of FT and 250 mg/kg of TdR at or below the LD10 dose of FT resulted in an increase in the antitumor activity to a % T/C of 217 (daily, Days 1–9) and 188 (daily, Days 1, 5, and 9). The activity of FT administered simultaneously with TdR on Day 1 only (% T/C = 142) was lower than that for FT alone. Using a treatment schedule of Days 1, 5, and 9, a TdR/FT mol ratio of ≥ 2.0 seems necessary to achieve an increase in therapeutic value against P388 murine leukemia. This may explain the lack of increase in activity against P388 when 250 mg/kg of TdR was coadministered with FT on Day 1 only. FT alone or coadministered with 250 mg/kg of TdR was equally active against L1210 ascites tumor at doses up to the LD10 with daily treatments on Days 1, 5, and 9 and on Days 1–9; the doses of FT, however, were below those which cause lethargy.

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The ability of thymidine (TdR) to increase the antitumor activity and host toxic effects of 5-FU has been the subject of much research and discussion (1–6). Oral coadministration of ftorafur (FT) with uracil (7,8), and, more recently, with TdR (9), has also been demonstrated to increase the cytotoxicity of FT. These studies failed to compare equitoxic doses of the two treatments so that the efficacy of the combination could not be evaluated. Our results indicate that parenteral coadministration of TdR and FT increases the cytotoxicity of FT against P388 and L1210 ascites tumors and increases toxicity to the normal host. The antitumor activity of the combination occurs at doses of FT below those which result in lethargy to the host.

METHODS

Male BDF_1 mice (Simonsen Laboratories, Gilroy, CA) were used in each experiment. The mice were housed in plastic cages with wood chip bedding and

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[4]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* V. Bruce Grossie, Jr, PhD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

were allowed at least a 7-day acclimation period before use. They were given food and water ad libitum from the time of receipt throughout the experiment. FT was obtained from the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). TdR was purchased from Sigma Chemical Co (St. Louis, MO). Both drugs were administered ip as a saline solution at 0.1 ml/10 g of body weight, the combination being injected simultaneously via separate syringes.

Host Toxic Effects

Normal mice were pooled in a common cage, weighed, and randomly assigned to a treatment group so that the weight range of the six to eight mice in each group was ≤ 4 g. The average weight of the groups (22–24 g) was used to determine the volume of drug solution to administer per mouse. For multiple treatment schedules, the average weight of each group on the day of treatment determined the volume of drug solution to be administered. Controls were treated with normal saline. Treatment followed one of three schedules: (*a*) daily for nine consecutive treatments (qd 1–9), (*b*) daily every 4th day for three treatments (q4d × 3), and (*c*) 1 day only (qd × 1). Mice were observed immediately after treatment for lethargy and for 14 days after the last treatment for mortality. The LD10 and LD90 were determined by a log dose-probit analysis, the fit of the line and data points confirmed by the chi-square test (10).

Antitumor Activity

Mice were inoculated ip with 10^6 P388 or 10^5 L1210 murine leukemia cells from the laboratory stock. On Day 1 of tumor implant the mice were weighed individually, and assigned to groups of six to eight as in the toxicity studies. Treatment was initiated on Day 1 and followed one of three schedules: (*a*) consecutive daily treatments on Days 1–9, (*b*) daily treatments on Days 1, 5, and 9, and (*c*) Day 1 only. The volume of drug solution administered was determined as previously described. The experiment was terminated when all of the mice had died or at 45 days after tumor implantation. The % T/C was calculated from the mean survival time for the drug-treated mice (T) and the saline-treated animals (C).

RESULTS

Host Toxic Effects

Simultaneous administration with 250 mg/kg of TdR increases the toxicity of FT to normal mice (table 1). FT is more toxic in divided doses when administered in combination with TdR. The cumulative LD10 dose of FT administered alone increased from 680 mg/kg when only one treatment was given to 1290 and 1170 mg/kg when administration was daily every 4th day for three treatments and daily for nine consecutive treatments, respectively, while that for FT administered simultaneously with 250 mg/kg of TdR decreased from 630 mg/kg for a single treatment to 186 and 117 mg/kg when the combination was administered daily every 4th day for three treatments and daily for nine consecutive treatments. Lethargy was observed in groups of mice treated with daily FT doses ≥ 150 mg/kg. Coadministration with TdR did not affect this toxic effect. TdR administered alone at a dose of 250 mg/kg was not toxic to normal mice.

Antitumor Activity

FT was inactive (% T/C < 125) against ascites P388 when administered alone in multiple-dose regimens (table 2). Simultaneous administration of 250

TABLE 1.—Toxicity of FT alone and in combination with 250 mg/kg of TdR to normal male BDF_1 mice

Treatment	Schedule of administration	Toxic dose (mg/kg/day)	
		LD10	LD90
FT	qd × 9	130	190
FT + TdR*		13	19
FT	q4d × 3	430	710
FT + TdR*		62	170
FT	qd × 1	680	1150
FT + TdR*		630	1100

*FT and TdR (250 mg/kg) were administered simultaneously ip, via separate syringes. TdR was not toxic at this dose.

TABLE 2.—Antitumor activity of FT and FT in combination with 250 mg/kg of TdR against P388 murine leukemia

Dose (mg/kg/day)*		Schedule of administration	No. of mice†	Survival		
FT	TdR			Mean ± SD‡	% T/C§	45-day survivors
10–150	0	Daily, Days 1–9	6–12	—	<125	—
5	250		28	14.7 ± 1.2	141	—
10	250		31	19.4 ± 1.5	188	1
12.5	250		19	16.6 ± 3.2	161	—
15.0	250		13	12.8 ± 1.6	123	—
17.5	250		13	11.2 ± 1.2	108	—
0	250		12	9.8 ± 0.6	96	—
50–500	0	Days 1, 5, 9	6–8	—	<125	—
37.5	250		12	16.7 ± 2.3	158	—
50	250		24	20.9 ± 3.5	195	—
62.5	250		12	23.0 ± 3.7	217	—
75	250		24	19.0 ± 4.5	179	—
87.5	250		12	18.4 ± 4.9	174	—
100	250		18	13.8 ± 2.1	129	—
112.5	250		12	14.8 ± 2.1	139	—
0	250		18	10.2 ± 1.0	94	—
500	0	Day 1 only	6	10.0 ± 2.1	111	—
600	0		12	12.5 ± 2.7	137	—
700	0		12	13.9 ± 1.4	153	—
800	0		12	12.0 ± 2.5	132	—
300	250		6	9.8 ± 1.2	109	—
400	250		12	11.9 ± 3.0	131	—
500	250		12	12.9 ± 4.0	142	—
600	250		12	11.5 ± 3.5	126	—

*FT and TdR were administered simultaneously ip via separate syringes at 0.1 ml/10 g of body weight.

†Total No. of mice/dose; includes the results from 2–4 experiments of 6–8 mice/dose for multiple-dose schedules and 1–2 experiments of 6–8 mice/dose for single-dose regimens.

‡Values = days (mean ± SD) for pooled data from all experiments at the respective dose.

§Calculated from the mean survival of drug-treated and saline-treated tumor-bearing BDF_1 mice. Mean survival of the saline-treated controls varied from 9.0 ± 1.1 to 11.0 ± 1.6 days after ip inoculation of 10^6 cells.

mg/kg of TdR potentiated the antitumor activity of FT. The peak activity occurred at or below the LD10 dose for the same number of treatments for normal mice. No benefit in antitumor activity could be ascribed in increasing the number of doses, although, as the number of treatments increased, the same antitumor activity was elicited with a lower daily dose of FT.

A single, high dose of FT alone, equal to the LD10 for this schedule, was moderately active against P388 leukemia (table 2); simultaneous treatment with 250 mg/kg of TdR did not enhance the antitumor activity of FT when administered up to the LD10 for normal mice. TdR alone at a dose of 250 mg/kg had no antitumor activity (table 2).

Fujii et al (7) reported that uracil increases the antitumor activity of oral FT and that the maximum increase in activity occurs at a uracil/FT mol ratio of 4. In our studies the TdR/FT mol ratio was ≥ 4 for FT doses at or below the 50-mg/kg treatment administered daily on Days 1, 5, and 9, the dose that demonstrated the second highest antitumor activity. The highest antitumor activity using this schedule occurred at an FT dose of 62.5 mg/kg, for which the TdR/FT mol ratio was 3.43. The TdR/FT mol ratio for the doses of FT that were administered on Day 1 ranged only from 0.60 for 400 mg/kg to 0.34 for 600 mg/kg.

To determine if the TdR/FT mol ratio was important with parenteral treatment, the daily on Days 1, 5, and 9 treatment schedule against P388 was chosen. Simultaneous administration of TdR had little effect on the antitumor activity of FT when the TdR/FT mol ratio was < 2.0 (table 3). The best ratio used was 4 when 50 mg/kg of FT was administered and 3 when 62.5 mg/kg was administered.

The effect of TdR on the antitumor activity of FT was additionally studied with ascites L1210 murine leukemia using multiple treatment schedules. The antitumor activity of FT against L1210 was not improved by simultaneous treatment with TdR (table 4) when the two regimens were compared at equitoxic doses (LD10). The antitumor activity against L1210, however, did occur at a lower daily dose of FT when administered simultaneously with TdR in both schedules.

TABLE 3.—Antitumor activity of FT in combination with varying mol ratios of TdR against P388 murine leukemia

Dose (mg/kg/day)*				Survival	
FT	TdR	TdR/FT mol ratio	No. of mice†	Mean ± SD (days)	% T/C‡
50	15	0.25	6	10.8 ± 1.5	105
	30	0.50	6	11.7 ± 1.5	117
	61	1.0	6	11.0 ± 2.1	107
	121	2.0	13	15.2 ± 1.6	148
	182	3.0	7	15.7 ± 2.0	153
	242	4.0	7	17.9 ± 2.0	174
62.5	76	1.0	7	12.7 ± 1.1	123
	152	2.0	7	16.3 ± 2.6	158
	227	3.0	7	21.4 ± 1.8	202
	303	4.0	7	17.6 ± 3.6	176

*FT and TdR were administered simultaneously ip via separate syringes daily on Days 1, 5, and 9.

†Total No. of mice/dose; includes the results from 1–2 experiments of 6–7 mice each.

‡Calculated from mean survival of drug-treated and saline-treated tumor-bearing BDF_1 mice. Mean survival of the saline-treated controls was 10.3 ± 1.3 days after ip inoculation of 10^6 cells.

TABLE 4.—Antitumor activity of FT and FT in combination with 250 mg/kg of TdR against L1210 murine leukemia

Dose (mg/kg/day)*				Survival	
FT	TdR	Schedule of administration	No. of mice	Mean ± SD (days)	% T/C†
50	0	Daily, Days 1–9	7	8.6 ± 0.8	102
80	0		7	10.3 ± 1.4	122
130	0		7	13.3 ± 1.4	158
5	250		7	10.6 ± 1.0	126
8	250		7	12.3 ± 0.8	146
13	250		7	13.7 ± 1.8	163
200	0	Days 1, 5, 9	7	11.1 ± 0.9	133
300	0		7	14.6 ± 0.2	174
400	0		6	12.0 ± 0.8	143
20	250		7	11.4 ± 1.0	136
40	250		7	14.1 ± 0.3	168
60	250		6	14.7 ± 1.0	175

*FT and TdR were administered simultaneously ip via separate syringes.

†Calculated from mean survival of drug-treated and saline-treated tumor-bearing BDF_1 mice. Mean survival of the saline-treated controls was 8.4 ± 0.7 days after ip inoculation of 10^5 cells.

DISCUSSION

The ability of TdR to increase the cytotoxicity of 5-FU has been documented (1–6), although the therapeutic index of the combination was significantly increased for one of four tumors studied (ascites P388) (1).

The current results demonstrate that FT alone is inactive against ascites P388 murine leukemia except when administered at high, single doses; even at these doses the activity is lower than when FT is administered with TdR using multiple treatment schedules. These data agree with those of Garibjanian et al (11) who demonstrated that FT had no activity against P388 when administered daily on Days 1, 5, and 9 or on Days 1–9. The LD10 for mice reported herein is approximately the same as that reported by Johnson et al (12).

When TdR was administered simultaneously with FT following daily treatments on Days 1, 5, and 9 and on Days 1–9, the cytotoxicity to ascites P388 leukemia was increased. The failure of 250 mg/kg of TdR to increase the antitumor activity of FT given on Day 1 only was unexpected, but may be explained by the low TdR/FT mol ratio that results with FT is administered at high doses; a dose of

TdR high enough to give a TdR/FT mol ratio of at least 2.0 is apparently necessary for FT to be active against P388 leukemia. The dose of 250 mg/kg of TdR given in combination with the higher, single doses of FT may, therefore, not be sufficient to increase the cytotoxicity to the host or tumor.

Simultaneous administration of FT and TdR did not increase the antitumor activity against ascites L1210 leukemia over that of FT alone when both treatments were compared at equitoxic doses (LD10). Lethargy, however, was consistently noted in mice treated with daily FT doses ≥ 150 mg/kg. The highest activity against L1210 leukemia when FT was administered alone in multiple doses occurred at doses which were near or above 150 mg/kg, while the dose of FT administered with 250 mg/kg of TdR was much lower.

Simultaneous treatment with FT and TdR increases the cytotoxicity of FT to the host and to P388 and L1210 murine leukemia. When compared at equitoxic doses, TdR increased the antitumor activity of FT toward P388 leukemia only; TdR did, however, reduce the dose of FT required to show antitumor activity toward P388 and L1210 leukemias to a dose which would not cause lethargy in the host. This may be of clinical importance when CNS toxicity is dose-limiting. It remains to be determined whether or not TdR alters the hematologic or gastrointestinal toxicity of FT.

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Human Myocardial Morphologic and Functional Changes in the First 24 Hours After Doxorubicin Administration[1]

Brief Reports and Preliminary Communications

Donald V. Unverferth,* Raymond D. Magorien, Barbara P. Unverferth, Robert L. Talley, Stanley P. Balcerzak, and Nobuhisa Baba[2,3]

SUMMARY

The purpose of this study was to document early structural changes in the human heart after the initial administration of doxorubicin. Endomyocardial biopsies were performed at baseline and at 4 and 24 hours after doxorubicin administration to 13 patients. Morphometric analysis of electron micrographs (× 31,200) quantitated mitochondrial and tubular sizes (sarcoplasmic reticulum and T tubules). The mitochondrial size increased from 0.25 ± 0.01 μ^2 ($\bar{X}$ ± SE) at baseline to 0.27 ± 0.03 μ^2 (not significant [NS]) at 4 hours and 0.30 ± 0.03 μ^2 (NS) at 24 hours. The tubular size increased from 2.24 ± 0.13 × 10^4 nm^2 at baseline to 2.60 ± 0.26 × 10^4 nm^2 ($P < 0.05$) at 4 hours and 2.46 ± 0.29 × 10^4 nm^2 (NS) at 24 hours. Micrographs analyzed for nuclear changes showed nucleolar contraction and segregation of granular and fibrillar components. These changes were noted in five of ten patients at 4 hours and in eight of 13 patients at 24 hours. Serial echocardiographic and systolic time interval determinations of left ventricular function demonstrated a significant improvement at both 4 and 24 hours. In conclusion, doxorubicin has its most significant effect on tubular structures. Left ventricular function improved during this time despite these findings.

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Doxorubicin is an effective cancer chemotherapeutic agent which often induces dose-related cardiomyopathy. The mechanisms of doxorubicin cardiotoxicity are not known. The purpose of this study was to closely investigate the myocardial morphologic and functional changes in patients during the first 24 hours after the initial administration of doxorubicin to define which subcellular structures were affected. Such knowledge may help to reveal the pathogenesis of doxorubicin cardiotoxicity.

METHODS

Patients

Thirteen patients received doxorubicin-containing therapy for the treatment of their malignancies (breast, seven patients; lung, three; sarcoma, one; lymphoma, one; and stomach, one). There were nine females and four males; the mean age was 47 years (range, 28–58). None of the patients had received prior chemotherapy or radiation therapy and the pretreatment cardiac history and physical examination were normal. Doxorubicin was given at a single dose of 30–60 mg/m^2 ($\bar{X}$ = 44) as an iv injection over a 5-minute period; no other chemotherapeutic agent was administered until after the 24-hour study period.

The endomyocardial biopsy procedure was performed according to the standard technique (1). Biopsy procedures were performed immediately prior to and 4 and 24 hours after the iv injection of doxorubicin in nine patients. Four patients had just two procedures, one before and one 24 hours after doxorubicin. At least two pieces of tissue were removed from the right side of the interventricular septum during each procedure. The specimens were submerged in 2% glutaraldehyde immediately on removal. Each tissue sample measured 1–2 mm^3 in size and weighed 2–5 mg. All tissue was handled in the same manner. Mitochondrial and tubular sizes

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[2]Departments of Medicine (*D. V. Unverferth, R. D. Magorien, R. L. Talley,* and *S. P. Balcerzak*) and Pathology (*B. P. Unverferth* and *N. Baba*), Ohio State University College of Medicine, Columbus.

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**Reprint requests to:* Donald V. Unverferth, MD, Department of Medicine, Ohio State University College of Medicine, 657 Means Hall, 466 W Tenth Ave, Columbus, OH 43210.

were evaluated by the method of Weibel (2) and as previously reported by our laboratory (3).

The electron microscope was also used to scan the myocardial nuclear structures of each biopsy specimen. Representative micrographs were taken at various magnifications (× 7000, × 12,000, and × 30,000).

Echocardiograms and systolic time intervals were performed before and 4 and 24 hours after the injection of doxorubicin. The echocardiographic measurement used for the determination of left ventricular function was the %ΔD and was defined as:

$$\%\Delta D = \frac{\text{end diastolic diameter} - \text{end systolic diameter}}{\text{end diastolic diameter}} \times 100.$$

Technically satisfactory echocardiograms were obtained in 12 of the 13 patients. The pre-ejection period/left ventricular ejection time (PEP/LVET) was used as the systolic time intervals measure of left ventricular performance (4).

Data were analyzed using Student's t test for paired observations. Any probability > 0.05 was considered to be not significant.

RESULTS

The average mitochondrial area at baseline was $0.25 \pm 0.01\ \mu^2$ ($\bar{X} \pm$ SE) and this increased to $0.27 \pm 0.03\ \mu^2$ 4 hours after doxorubicin, and even further to $0.30 \pm 0.03\ \mu^2$ at 24 hours. These changes were not significant ($P > 0.05$). The swelling of the tubular structures was significant at 4 hours and decreased at 24 hours. The average tubular area was $2.24 \pm 0.13 \times 10^4\ nm^2$ at baseline; it increased to $2.60 \pm 0.26 \times 10^4\ nm^2$ at 4 hours ($P < 0.05$) and decreased to $2.46 \pm 0.29 \times 10^4\ nm^2$ at 24 hours (fig 1). Figure 2 (a and b) presents the electron micrograph findings from a typical patient.

The nucleolar changes are shown in figure 3 (a–d). Before doxorubicin administration nucleoli showed the normal nucleonema structure (fig 3a). After doxorubicin, an occasional nucleus demonstrated nucleolar contraction as well as segregation of granular and fibrillar components. These changes were noted in five patients at 4 hours and in eight patients at 24 hours. Most nucleoli demonstrated no change, however, and they resembled the baseline status.

The results of the noninvasive testing are shown in figure 4. Both the PEP/LVET of the systolic time intervals and the echocardiographic %ΔD demonstrated significantly improved left ventricular function at 4 and 24 hours compared with baseline.

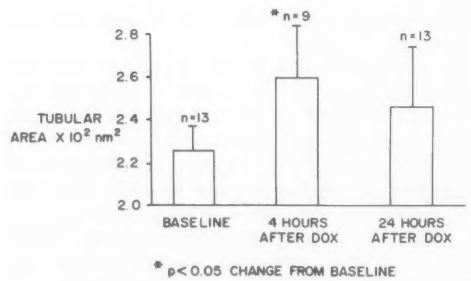

FIGURE 1.—Average ($\bar{X} \pm$ SE) size of the tubular structures (sarcoplasmic reticulum + T tubules) is shown. These structures were significantly enlarged from baseline at 4 hrs.

DISCUSSION

The pathologic changes of continuous doxorubicin therapy (cumulative dose > 240 mg/m²) include swelling of sarcoplasmic reticula and mitochondria, formation of cytoplasmic vacuoles, myofibrillar loss, and chronic nuclear degeneration (5). The acute pathologic changes have not been previously reported in human heart tissue. This study has demonstrated that the acute changes were similar to chronic changes. Although nucleolar alterations did not occur in all patients, there was some degeneration with segregation of granular and fibrillar components. Swelling of the tubular structures and mitochondria was also associated with doxorubicin. The sarcoplasmic reticulum and T tubules were significantly increased in size at 4 hours; the swelling was somewhat diminished by 24 hours. The increase in mitochondrial size did not achieve statistical significance. Thus, the most remarkable morphologic alteration in patients after a pharmacologic dose of doxorubicin was in the tubular structures.

Continuous doxorubicin therapy (large cumulative doses) results in a depression of myocardial function as measured by systolic time intervals (6), echocardiograms (7), and radionuclide angiography (8). The cardiac function tests performed in the first 24 hours after the initial dose of doxorubicin demonstrated a significant improvement (fig 4). This improvement occurred despite the morphologic changes noted above. The improved function in the first 24 hours could be anticipated because low-dose doxorubicin in vitro increased myocardial peak developed force and the rate of tension rise (9). Large-dose doxorubicin in vitro studies and supraphar-macologic in vivo studies have caused deterioration of myocardial function (9,10). Thus, pharmacologic

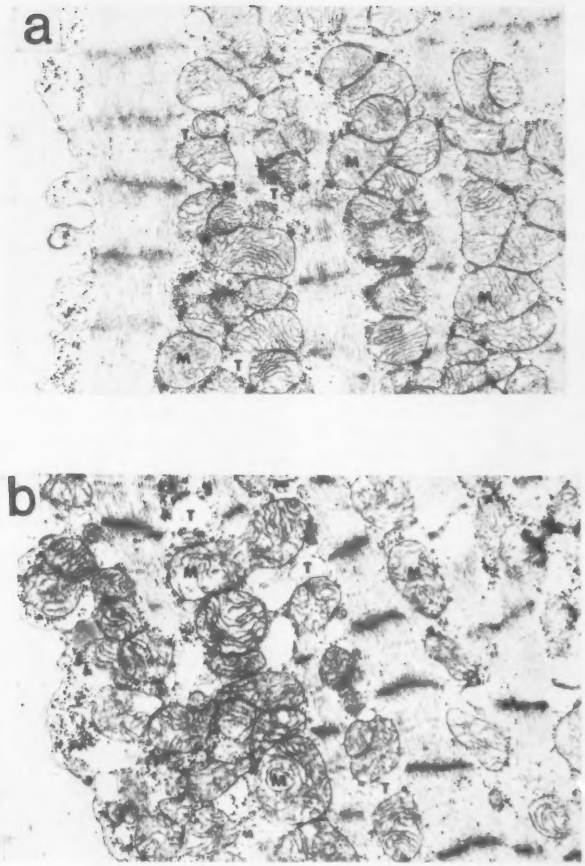

FIGURE 2.—**a.** Predoxorubicin biopsy specimen (× 31,200) demonstrates normal size and structure of mitochondria (M) and tubules (T). **b.** 24-hr biopsy from the same patient demonstrates swelling of mitochondria (M) and tubules (T).

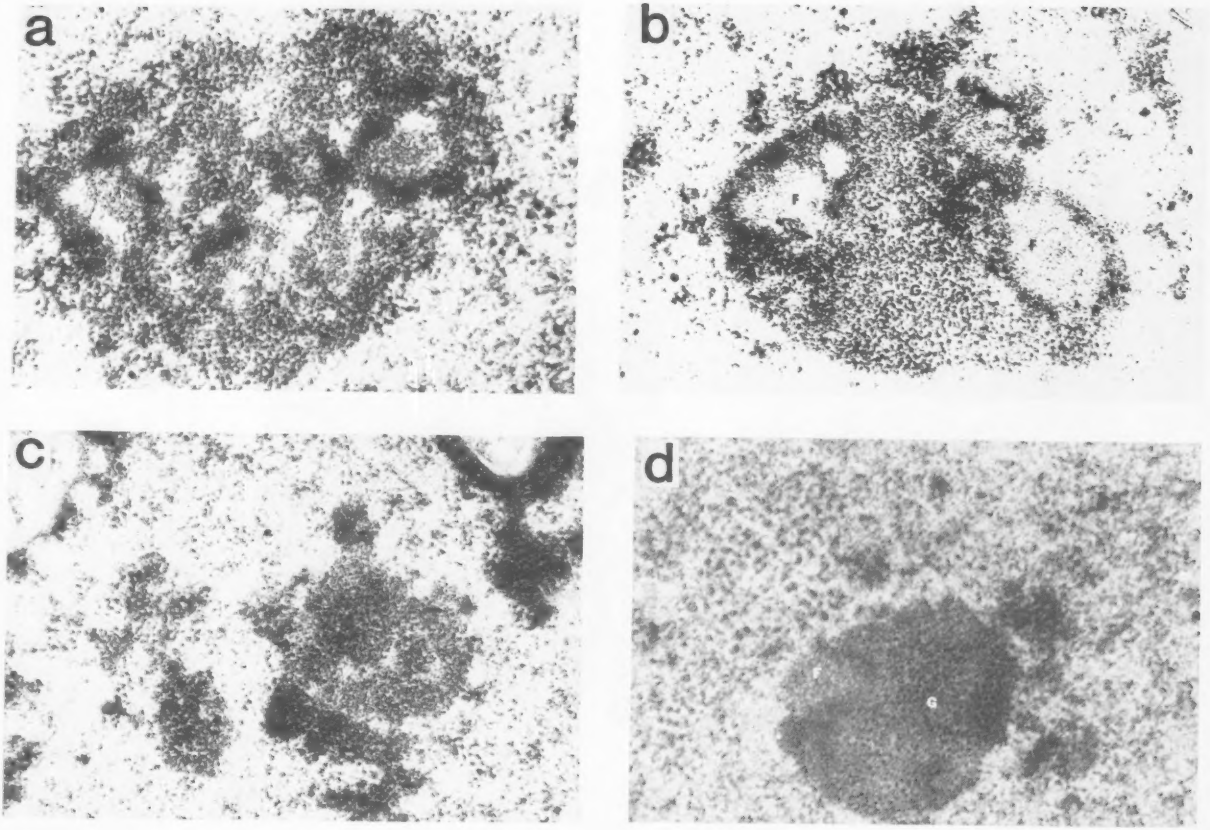

FIGURE 3.—**a.** Normal predoxorubicin nucleolus. Granular and fibrillar components are randomly arranged in a trabecular structure. **b.** 4 hrs after doxorubicin, nucleolus appears compact. There is a distinct segregation of granular (G) and fibrillar (F) components. **c.** 24 hrs after doxorubicin, the nucleolus appears compact. Trabecular structure is no longer evident. **d.** 24 hrs after doxorubicin, nucleolus is compact and shows a distinct segregation of granular (G) and fibrillar (F) components (× 75,000).

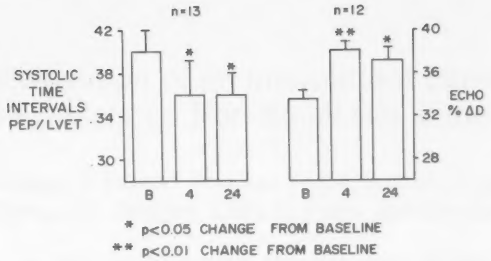

FIGURE 4.—There was a significant improvement of myocardial function both 4 and 24 hrs after doxorubicin. PEP/LVET ratio improved, as did the %ΔD of the echocardiogram (Echo).

doses of doxorubicin administered to patients mimic low-dose in vitro studies.

In conclusion, pharmacologic doses of doxorubicin significantly damage tubular structures in the first 24 hours. There are also some changes of nuclear and mitochondrial structures, yet overall cardiac function is maintained. We hypothesize that repeated insults to these subcellular structures eventually lead to loss of cellular function and impaired myocardial performance.

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Evaluation of an Intermittent Schedule of Mitolactol in Advanced Non-Small Cell Lung Cancer[1,2]

Brief Reports and Preliminary Communications

Robert T. Eagan,* Stephen Frytak, William C. Nichols, James N. Ingle, Edward T. Creagan, Larry K. Kvols, and Douglas T. Coles[3]

In 1977, we reported a phase II study of dianhydrogalactitol (DAG) in patients with advanced non-small cell lung cancer (NSCLC) (1). DAG, an iv preparation, is the main conversion reaction product of the treatment of mitolactol (DBD), an oral agent, by mild alkali or human serum (2). Wilson et al (3), using a continuous oral schedule, reported a regression rate of 13%, 24 of 179 patients with advanced lung cancer, while Phillips and Brook (4) noted no regressions in similar patients using a similar schedule but a lower drug dose. Recently, Tormey et al (5) devised a 10-day intermittent oral schedule for DBD which achieved some regressions in patients with breast cancer, many of whom had had prior chemotherapy. They recommended a dose of 180 mg/m²/day for 10 days repeated every 28 days in patients with prior chemotherapy exposure. Because a phase II study of the oral intermittent schedule of DBD had not been conducted in lung cancer patients in the United States and because antitumor activity was noted in patients with prior alkylator exposure, we evaluated DBD in patients with advanced NSCLC.

MATERIALS AND METHODS

Sixty-three patients with advanced NSCLC were entered in the study and all were evaluable. To be eligible for study, each patient was required to have histologic or cytologic evidence of NSCLC, measurable or evaluable disease, normal wbc and platelet counts, a serum creatinine < 2.0 mg/dl, no elevation of the direct serum bilirubin, and an Eastern Cooperative Oncology Group performance score (ECOG PS) < 4. All patients had to have finished any prior chemotherapy at least 3 weeks earlier (6 weeks for mitomycin or a nitrosourea).

Patients with measurable disease were assessed for response according to standard criteria. Patients with evaluable, but not measurable, disease were assessed according to the criteria of Eagan et al (6). A partial regression (PR) of measurable disease was considered to be equivalent to a regression (R) of evaluable disease.

Two dose levels of DBD were studied. Patients ≤ 70 years old with an ECOG PS of 0–2 and no prior chemotherapy were treated at a dose of 225 mg/m²/day orally for 10 consecutive days. Patients > 70 years old or with an ECOG PS of 3 or with prior chemotherapy were treated at a dose of 175 mg/m²/day orally for 10 consecutive days. Initially the re-treatment interval was scheduled for 4 weeks but later was extended to 5 weeks routinely in the older patients and in patients receiving the higher dose level because of late and cumulative bone marrow toxicity. DBD was given as a single daily dose at bedtime.

At the time of scheduled re-treatment, doses were adjusted only on the basis of previous nadirs. If mild myelosuppression (wbc count < 4000 but ≥ 3000 cells/mm³ or platelet count < 130,000 but ≥ 100,000 cells/mm³) was still present, an additional week was added to the re-treatment intervals. If blood counts were less than the values indicated above, treatment was delayed.

Of the 63 patients treated, 51 were treated at the 175-mg/m² level and 12 at the 225-mg/m² level. The majority of patients (50%) had adenocarcinoma (ACA), 31% had squamous cell carcinoma (SQC), and 19% had large cell carcinoma (LGC). Most patients had an ECOG PS of 2–3 (58%) and had received prior chemotherapy (64%). The patients were somewhat old (median age, 62 years; range, 32–81) and predominantly male (75%). The pretreatment characteristics of the patients are shown in table 1.

RESULTS

Response data.—Five of the 63 patients (8%) had tumor regressions (one complete regression, two PRs, and two Rs). By cell type, regressions were as follows: ACA, two of 31 patients (6%); SQC, two

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[3]Division of Medical Oncology, Mayo Clinic, Rochester, MN.

**Reprint requests to:* Robert T. Eagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55901.

of 20 patients (10%); and LGC, one of 12 patients (8%). Regressions occurred in two of 41 patients (5%) who had had prior chemotherapy and in three of 22 (14%) who had had no prior chemotherapy. Two of 27 patients (7%) having ECOG PS 0–1 and three of 36 (8%) having ECOG PS 2–3 had regressions. By DBD dose, regressions were as follows: 175 mg/m², three of 51 patients (6%); and 225 mg/m², two of 12 patients (17%).

Response durations and times to disease progression.—The two ACA regressions were both Rs lasting 145 and 168 days. The one LGC regression was a PR lasting 330 days. One of the two SQC regressions was a PR of 65 days and the other was a complete regression lasting 288 days. All regressions were calculated from the first day of treatment. Disease progression occurred in 43 patients (67%) after the first course of DBD and in 11 (17%) after the second course. Twenty-one patients received two or more courses of DBD (maximum: six).

Survival.—At present, 39 of the 63 patients (62%) have died (53% of those with ACA, 75% with LGC, 65% with SQC). Median survival data were obtained from Kaplan-Meier curves. The median survival for all 63 patients was 100 days (range, 12–277+). By cell types, the median survival times were as follows: patients with ACA, 137 days; with SQC, 89 days; and with LGC, 77 days.

Toxicity

Myelosuppression was the only toxic reaction of note. It occurred in 45% of the initial courses and in 60% of all treatment courses and was more often thrombocytopenia than leukopenia. This toxic effect by dose of DBD, treatment course, and prior chemotherapy is shown in table 2. The degree and frequency of myelosuppression was not clearly related to age, sex, ECOG PS, or liver status. More frequent and severe myelosuppression was seen at the higher DBD dose.

The median time to wbc count nadir was 20 days (range, 5–39), with 38% of nadirs occurring on Day 28 or later. The median time to platelet count nadir was somewhat later at 28 days (range, 4–41), with 20% of nadirs occurring on Day 35 or later.

The assessment of cumulative toxicity was difficult because only eight patients (four each at 175 and 225 mg/m²) were treated two or more times at the same dose level. Evidence in support of cumulative myelosuppression included a decrease in median wbc and platelet count nadirs between first versus subsequent treatments (6625 to 4050 cells/mm³ and 140,000 to 90,000 cells/mm³, respectively) and an increased frequency of leukopenia (one of eight first courses vs four of eight later courses) and thrombocytopenia (four of eight first courses vs seven of eight later courses).

DISCUSSION

In our earlier trial of DAG (1), the regression rate reported was 10% (six of 62 patients) versus 8% (five of 63 patients) in this study of DBD. Among patients with no prior chemotherapy, the regression rate for DAG was 11% (six of 55 patients) versus

TABLE 1.—Pretreatment patient characteristics by cell type and dose of DBD

	ACA		LGC		SQC		Total	
	175 mg/m²	225 mg/m²	175 mg/m²	225 mg/m²	175 mg/m²	225 mg/m²	175 mg/m²	225 mg/m²
Patients treated	27*	4	10	2	14	6	51	12
No prior chemotherapy	4	4	2	1	6	6	12	11
ECOG PS								
0–1	11	2	4	1	4	5	19	8
2–3	16	2	6	1	10	1	32	4
Sex								
Male	20	2	8	1	11	6	39	9
Female	7	2	2	1	3	0	12	3
Age (yrs)								
Median	62	55	61	43	69	60	62	55
Range	42–77	46–66	32–76	42–44	49–81	37–69	32–81	37–69
Indicator lesions								
Measurable	8	1	6	1	5	1	19	3
Evaluable	19	3	4	1	9	5	32	9

*Unless specified otherwise, values = No. of patients.

TABLE 2.—% of patients with hematologic toxicity by dose, course, and prior chemotherapy

	175 mg/m²				225 mg/m²	
	Prior chemotherapy		No prior chemotherapy		No prior chemotherapy	
	First course	All courses	First course	All courses	First course	All courses
Wbc count nadir (cells/mm³)						
≥ 4000	73%	61%	69%	69%	73%	18%
< 4000 to ≥ 1500	27%	39%	23%	23%	18%	64%
< 1500	0	0	8%	8%	9%	18%
Platelet count nadir (cells/mm³)						
≥ 130,000	64%	47%	54%	54%	55%	18%
< 130,000 to ≥ 50,000	24%	41%	38%	38%	27%	55%
< 50,000	12%	12%	8%	8%	18%	27%

13% (three of 23 patients) for DBD. The regression rates for DAG and DBD are quite similar and comparable to those reported for doxorubicin (7) and cyclophosphamide (8) in more recent studies. The regression rate for the intermittent dose schedule of DBD in this study is also quite consistent with that reported by Mischler et al (9) with DBD used in a variety of dose schedules for lung cancer.

The myelosuppression from DBD was similar in degree and frequency to that from DAG. Thrombocytopenia was more frequent and severe than leukopenia. Since 15% of patients experienced severe myelosuppression (wbc count nadir < 1500 cells/mm³ or platelet count nadir < 50,000 cells/mm³) and because of the suggestion of cumulative hematologic toxicity, we feel that the doses studied were adequate for this phase II assessment of DBD. A 5-week re-treatment interval is recommended should other phase II evaluations of intermittent-dose DBD be contemplated.

DBD does not seem worthy of further study in patients with advanced NSCLC who have had prior chemotherapy.

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Erythrocyte Osmotic Fragility in Patients Receiving Hyperthermia With and Without Chemotherapy[1]

Brief Reports and Preliminary Communications

Jacqueline Whang-Peng,* David Eric Lees, William H. Schuette, Rosalie Smith, Joan M. Bull, and Vincent T. DeVita, Jr[2,3]

SUMMARY

The osmotic fragility of erythrocytes was studied in 24 patients receiving whole-body hyperthermia, with and without concomitant chemotherapy, for 2–4 hours as an adjuvant to chemotherapy for advanced metastatic disease. No alteration in osmotic fragility of erythrocytes was observed with whole-body hyperthermia.

[Cancer Treat Rep 65:1103–1104, 1981]

Whole-body hyperthermia to 42°C is currently being tested as an adjuvant to chemotherapy in the treatment of advanced metastatic disease. The feasibility and toxicity of this potential adjuvant treatment were summarized in a previous report (1). Tumor cells have a greater thermal sensitivity than do normal cells (2). In addition, heat and chemotherapeutic agents act synergistically to increase cell kill (3). Both acute hemolytic anemia and increased erythrocyte osmotic fragility have been observed following extensive thermal injury (4,5). These changes are thought to be due to the direct effect of heat on the erythrocyte. In this study, we examined the osmotic fragility of erythrocytes from 24 patients undergoing whole-body hyperthermia, with or without concomitant chemotherapy, to determine if thermal exposure increases fragility.

[1]Received Oct 3, 1980; revised Feb 17, 1981; accepted Mar 4, 1981.

[2]Division of Cancer Treatment, National Cancer Institute (*J. Whang-Peng, R. Smith, J. M. Bull,* and *V. T. DeVita, Jr*), Anesthesiology Service, Clinical Center (*D. E. Lees*), Biomedical Engineering and Instrumentation Branch, Division of Research Services (*W. H. Schuette*), National Institutes of Health, Bethesda, MD.

[3]We thank Ms. Minna Feld for technical assistance in performing the osmotic fragility determinations, Dr. Jerome Donlon for suggestions on presenting the data, and Mrs. Turid Knutsen for manuscript preparation.

**Reprint requests to:* Jacqueline Whang-Peng, MD, Bldg 10, Rm 12N-226, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

MATERIALS AND METHODS

Twenty-four patients (16 males and eight females) were studied after giving informed consent to this institutionally approved protocol; the mean age was 51 years (range, 23–61). Oncologic diagnoses were: malignant melanoma (nine cases); adenocarcinoma of the colon (six); adenocarcinoma of the stomach, carcinoma of the adrenal gland, squamous cell carcinoma of the lung, squamous cell carcinoma of the maxillary sinus, giant cell tumor of the femur, fibrohistiocytoma, and chondrosarcoma (one each); and leiomyosarcoma (two). Initially, all patients underwent 2–4 hours of hyperthermia alone; a repeat treatment followed 1 week later, with either chemotherapy plus hyperthermia or hyperthermia alone. Chemotherapy was administered to 17 of the 24 patients when the core temperature exceeded 41.5°C during the induction of hyperthermia. Seven of the patients received iv doxorubicin (10–30 mg/m^2) and ten received oral methyl-CCNU (75–200 mg/m^2). The body temperature was elevated by a specially designed, low volume, high flow, heated-water perfusion suit that covered the entire body with the exception of the head and neck (1). The core temperature was maintained at a plateau of 41.8°C (± 0.1°C variation) during the treatment period (4 hours with no chemotherapy, 2 hours with chemotherapy). Venous blood samples for osmotic fragility testing were drawn prior to the induction of hyperthermia (pre), upon attaining the minimum therapeutic temperature of 41.5°C (zero time), at the end of the treatment plateau (2–4 hours), and the next day (24 hours).

Osmotic fragility test.—The standard osmotic fragility test as described by Dacie and Lewis (6) was used. Blood samples (2×10^{-2} ml) were added to a standard range of hypotonic saline solutions. The tubes were allowed to stand at room temperature (20°C) for 30 minutes, then remixed and centrifuged for 5 minutes at 1200–1500 × *g*. Duplicate blood samples were also incubated at 37°C for 24 hours prior to testing. The amount of hemolysis in

each tube was then compared with that in the 100% lysis tube (0.1% NaCl), using a photoelectric colorimeter or photometer with a yellow-green filter. The osmotic fragility curves in all the samples were plotted and the concentration that produced 50% hemolysis was noted.

RESULTS

Osmotic fragility data for the three groups (repeat hyperthermia alone, hyperthermia plus methyl-CCNU, and hyperthermia plus doxorubicin) are summarized in figure 1. The entire fragility curve was determined with 12 dilution points over a range of 0.10%–0.85%. All osmotic fragility curves were sigmoidal and within the normal range. No significant changes in hemolysis were seen in any of the three groups as therapy progressed or during repeat exposure to hyperthermia with or without chemotherapy.

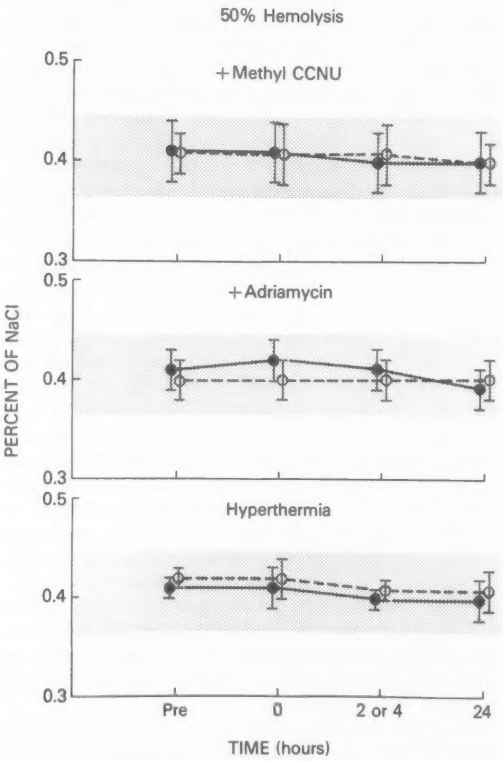

FIGURE 1.—Shaded area indicates normal range of sodium chloride concentrations that produces 50% hemolysis. Sampling times were: prior to induction of hyperthermia (pre), at start of plateau phase with temperature > 41.5°C (time 0), after 2 or 4 hrs of treatment (2 or 4 hrs), and next day (24 hrs). Solid lines = first hyperthermia treatment, dotted lines = second treatment, values = mean ± SE.

DISCUSSION

There are no published reports on erythrocyte fragility in persons receiving intentionally induced hyperthermia. In normal individuals, in vitro preincubation of blood samples for 24 hours produces a moderate increase in osmotic fragility (0.365%–0.445% to 0.425%–0.525% for 50% lysis). Our findings, however, do not demonstrate any effect of heat on osmotic fragility in patients undergoing whole-body hyperthermia for a brief exposure of 2–4 hours. This is further supported by our previous observation that no hemoglobin can be found in the urine of patients undergoing hyperthermia (1). The effect of prolonged hyperthermia on erythrocyte osmotic fragility, however, has not been elucidated. The seven patients receiving doxorubicin experienced the expected clinical toxic reactions, yet there was no increase in osmotic fragility over the previous treatment with hyperthermia alone. The ten patients who received methyl-CCNU failed to exhibit any of the clinical toxic reactions expected with the nitrosoureas, even with a dose of 250 mg/m^2, raising the possibility that the drug was not being absorbed in an active form. Therefore, the effect of methyl-CCNU, if any, on osmotic fragility remains uncertain.

In conclusion, no alteration in the osmotic fragility of erythrocytes was seen in 24 patients receiving whole-body hyperthermia for 2–4 hours, with or without concomitant chemotherapy.

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Doxorubicin and Cisplatin Therapy in Children With Neuroblastoma Resistant to Conventional Therapy: A Southwest Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

Ruprecht Nitschke,* Kenneth Starling, Victor K. S. Lui, and Jeanette Pullen [3,4]

SUMMARY

Fifteen children with metastatic neuroblastoma resistant to vincristine and cyclophosphamide were treated with two drugs which were known to be effective as single drugs against neuroblastoma. The drugs were given in courses every 3 weeks. Doxorubicin (50 mg/m² iv) was given on Day 1 and cisplatin (50 mg/m²) was administered on Day 2 as an 8-hour infusion, using a forced diuretic-hydration program. Three of the 15 children achieved partial or complete remission. Three children showed improvement. The other children either did not respond to the therapy or had progressive disease. The combination of doxorubicin and cisplatin given in the sequence outlined is no more effective than either drug given singly. The side effects of the drug combination were tolerable and were in keeping with previously described toxicity.

[Cancer Treat Rep 65:1105–1108, 1981]

Doxorubicin (ADR) and cisplatin have been shown to be effective as single agents in patients with neuroblastoma resistant to conventional therapy (1–5). In addition, the combination of these two drugs improved the results in patients with ovarian carcinoma beyond those achieved with cisplatin alone (6). The Pediatric Division of the Southwest Oncology Group (SWOG) investigated the combination therapy with the two agents in children with recurrent neuroblastoma. The doses of the two drugs were identical to those used for the treatment of ovarian cancer. However, ADR was given 24 hours prior to cisplatin, rather than on the same day. The schedule was modified because of the findings in $CD2F_1$ mice with advanced L1210 disease. Using the spleen colony assay technique, maximal reduction of L1210 cells occurred when ADR was administered 16–24 hours prior to cisplatin (7). The study was initiated in 1978 and was closed after 1 year.

METHODS

Patients with neuroblastoma who were between the ages of 1 and 21 years were eligible if they (*a*) had progressive disease, (*b*) were resistant to vincristine and cyclophosphamide, (*c*) had at least one measurable lesion not recently treated with radiation therapy, (*d*) had not received chemotherapy for 1 week prior to the therapy, and (*e*) had normal kidney function (serum creatinine < 1 mg/dl in children < 5 years old, serum creatinine < 1.2 mg/dl in older children, and BUN ≤ 30 mg/dl). A consent form in accordance with the regulations of the individual institution was required.

Patients were classified as good- or poor-risk. Good-risk patients were defined as those whose previous treatment had not included radiotherapy to the myocardium or to the major portion of the bone marrow and/or who had not received high doses of cyclophosphamide (≥ 40 mg/kg/day × 2 every 4 weeks). Poor-risk patients were defined as patients who had previously been treated with radiation therapy to the major portion of the bone marrow or to the myocardium and/or had received high doses of cyclophosphamide.

The two drugs were given every 3 weeks as fol-

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[3]Oklahoma Children's Memorial Hospital, Oklahoma City (*R. Nitschke*); Texas Children's Hospital, Houston (*K. Starling*); Emory University, Atlanta, GA (*V. K. S. Lui*); and Children's Hospital, Jackson, MS (*J. Pullen*).

[4]We thank Eva Hanes for secretarial assistance in preparing this manuscript.

**Reprint requests to:* Dr. R. Nitschke (7816), Pediatrics Oncology Group, Operations Office, 4386 Lindell Blvd, St. Louis, MO 63108.

lows: ADR (50 mg/m² iv) on Day 1 and cisplatin (50 mg/m²) for 8 hours on Day 2. Patients in the poor-risk group received ADR (40 mg/m² iv) on Day 1 and cisplatin (50 mg/m²) on Day 2. At the time of cisplatin infusion, a hydration and diuretic program was mandatory. This program was identical to that previously described, except that mannitol was not infused (3). After cumulative ADR doses of 450 mg/m² for good-risk patients and 350 mg/m² for poor-risk patients had been reached, treatment with cisplatin alone was continued. Drug modifications were made in case of myelosuppression, elevations of serum BUN and creatinine, liver or myocardial dysfunction, and gastroenteric symptoms.

Physical examination, cbc, and determination of serum BUN and creatinine were required weekly during the first 6 weeks of treatment and every 3 weeks thereafter, just prior to the start of therapy. In addition, liver function tests were required before each course of therapy. Evaluation of tumor mass by appropriate technique was performed every 3 months. The SWOG criteria for solid tumor responses were as follows: *complete remission (CR)*—complete disappearance of all measurable lesions; *partial remission (PR)*—regression of all tumors by ≥ 50%; and *improvement*—regression of all tumors by < 50% (one or more metastatic lesions may show a > 50% reduction in size).

RESULTS

Seventeen patients were entered in the study. Two were not included in this analysis because they had received prior ADR therapy. Two of the 15 patients were only partially evaluable because of loss to followup and parental refusal of further therapy. There were nine patients in the good-risk group and six in the poor-risk group. Their ages varied from 12 months to 10 years and 4 months, with a median of 5 years and 2 months. The interval between diagnosis and entrance in the study varied between 50 and 1130 days, with a median of 194 days. All patients had been previously treated with and were resistant to vincristine and cyclophosphamide. These drugs had been combined with BCNU in ten patients, with F3TdR and papaverine in two patients, and with DTIC in three. Nine of these patients had bony lesions. The children were in the study between 37 and 347 days, with a median of 91 days. They received between two and 14 courses, with a median of five. Two children achieved CR, one child achieved PR, three had improvement, and nine had disease progression. Both children who achieved a CR had metastatic disease only in the bone marrow at the time of entry in the study. The therapy of one child was discontinued at the request of the parents 21 days after the CR was achieved. The other child was in remission for 318 days when last evaluated. The child who achieved a PR had abdominal and mediastinal masses and neuroblastoma cells in the bone marrow. His remission lasted 204 days.

Toxic effects were evaluated in 15 children. Myelosuppression was primarily manifested by moderate to severe neutropenia (table 1). A neutrophil count nadir < 1000/μl was observed between courses in 26 of 64 courses given to the children. The therapy had to be delayed in 21 of these 64 courses for > 3 days because of slow recovery from the neutropenia. Moderate vomiting (less than six times) was observed in ten children after one or more courses, one child vomited more frequently, and four children had no gastrointestinal complaints. Ten children had normal serum BUN levels, four had levels of 21–40 mg/dl, and one child had a level of 56 mg/dl. Only two children had elevated serum creatinine levels: 1.8 and 4.1 mg/dl. Their abnormal values normalized after 8 and 15 days, respectively. The therapy was continued without recurrence of elevated serum BUN or creatinine. Two children had serum magnesium levels < 1.8 mg/dl without showing any clinical symptoms related to the low level.

DISCUSSION

The CR plus PR rate of the two-drug combination was 20%. ADR, given as a single agent to patients who had had prior therapy, produced response rates of 15%–65% (1,8,9), and cisplatin, given as a single agent, demonstrated a 17%–65% effectiveness (2–5). The failure to achieve an additive therapeutic effect by combining these two drugs may be due to the dose of cisplatin used and/or to the sequence in which these two drugs were given. The cisplatin dose chosen was based on its therapeutic success in ovarian tumor (6). In that study, the effective dose of cisplatin (50 mg/m² every 3 weeks) as a single agent was used in combination with ADR. This low dose was not tested in neuroblastoma patients; only higher doses (90–120 mg/m² given within 3 weeks) were used (2–5). It is possible that the neuroblastoma cells are less sensitive to cisplatin than the ovarian cancer cells, and for that reason the cisplatin dose in our study may have been too low. The sequence in which cisplatin and ADR were given was based on the results in $CD2F_1$ mouse experiments (7). Cisplatin may affect neuroblastoma cells in a different way. In a recent study, cisplatin produced an increase in the labeling index and a decrease in the mitotic index at 48 hours, in seven of

TABLE 1.—Occurrence rate of myelotoxicity in patients who had normal values prior to therapy

	Toxicity grade					
	0	I	II	III	IV	Total
	Ranges defining grades					
Hgb (g/dl)						
Patients < 2 yrs	≥ 10.0	9.0–9.9	7.0–8.9	5.0–6.9	< 5.0	
Patients > 2 yrs	≥ 11.0	10.0–10.9	7.0–8.9	5.0–6.9	< 5.0	
Granulocytes/μl	> 1500	1000–1500	500–999	250–499	< 250	
Platelets/μl	≥ 100,000	75,000–99,999	50,000–74,999	25,000–49,999	< 25,000	
	No. of patients					
Hgb	1	5	4	0	0	10
Granulocytes	1	3	3	4	4	15
Platelets	8	2	0	1	4	15

nine patients who had neuroblastoma in bone marrow (10). This finding was interpreted to suggest an accumulation of cells in S phase and a delay or depletion of cells in G_2 phase. Because ADR (35 mg/m^2) produced changes of the mitotic and labeling indices of neuroblastoma cells in seven of 11 patients, indicating cell lysis in S phase and a G_2 and G_1 block in five of them, the most promising program would be to use cisplatin first and to administer ADR 48 hours later (11). Another therapeutic approach would be to use the doses of ADR and cisplatin which are recommended when the drugs are given alone and to alternate these drugs every 3 weeks. To date, this approach has been used only in patients with nonmetastatic osteogenic sarcoma (12).

The toxic effects of the two-drug combination given to 46 adults with ovarian tumors consisted of protracted nausea and vomiting and bone marrow myelosuppression, which resulted in anemia (Hgb < 8 g/dl) in nine instances and in leukopenia (< 1000 wbcs/μl) in eight instances (13). Elevated serum creatinine values were observed in two patients. The children with neuroblastoma experienced similar toxic effects. However, gastrointestinal disturbances were less frequently observed. In addition, low serum magnesium values were noted in two children: a finding which has been reported to occur after cisplatin administration (2,5).

In summary, ADR given on Day 1 and cisplatin given on Day 2 every 3 weeks produced a low remission rate in patients with neuroblastoma resistant to conventional therapy. This therapeutic failure may have been caused by the schedule for administration of these two drugs and/or by the low dose of cisplatin. In the future, this combination should be re-evaluated by giving cisplatin first and ADR after 48 hours or by alternating both drugs every 3 weeks, using the dose recommended when the drugs are given as single agents.

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Phase II Study of Chlorozotocin in Leukemia and Other Hematologic Malignancies [1,2]

Brief Reports and Preliminary Communications

Santiago Pavlovsky,[3] Paul V. Woolley,[4,*] Guy Garay,[3] Cristina Scaglione,[3] Daniel Hoth,[4] and Philip S. Schein [4]

Chlorozotocin is a glucose-containing chloroethylnitrosourea that is relatively nonmyelosuppressive at doses that produce significant antileukemic activity in L1210 murine leukemia (1). In phase I trials (2,3), chlorozotocin has been shown to induce myelosuppression as a dose-limiting toxic effect at doses > 175 mg/m², but also to have antitumor activity at doses that are nonmyelosuppressive, eg, 120 mg/m² given every 6 weeks (4,5). The present phase II trial has tested chlorozotocin using the 120-mg/m² dose in leukemia and lymphoma patients. This dose was given either weekly or daily for 3 days to the leukemia patients, and every 6 weeks to the lymphoma patients.

MATERIALS AND METHODS

The study population consisted of 20 patients with relapsed acute leukemia (eight patients with acute myelocytic leukemia, 11 with acute lymphocytic leukemia, and one with chronic myelocytic leukemia in blast cell crisis) and 18 patients with chronic lymphocytic leukemia, lymphoma, or multiple myeloma. The median age was 20 years (range, 5–40). All patients had objectively measurable disease: either marrow infiltration, circulating tumor cells, or palpable adenopathy. All of the leukemia patients were resistant to daunorubicin, vincristine, prednisone, cytarabine, cyclophosphamide, 6-mercaptopurine, and methotrexate. None had received L-asparaginase. Five patients were resistant to induction therapy, 11 were in first relapse, two were in second relapse, and one was in fourth relapse. All of the lymphoma patients had received prior chemotherapy.

Chlorozotocin was administered iv at a dose of 120 mg/m². This dose was given 6 weeks to lymphoma patients and in a more intensive schedule (either weekly or daily for 3 days) to leukemia patients.

A complete response was defined as the total regression of all objective indications of disease, and a partial response was defined as a > 50% reduction in all objectively measurable disease, with no progression in other sites.

RESULTS

Thirteen patients with acute leukemia received weekly chlorozotocin and seven received the drug daily for 3 days (table 1). Of those patients receiving the weekly schedule, two of five with acute myelocytic leukemia and three of seven with acute lymphocytic leukemia had transient decreases in numbers of circulating blast cells. None, however, exhibited a bone marrow response, either in terms of overall cellularity or in terms of percent of blast cells (table 1). Six of the seven patients who received chlorozotocin daily for 3 days had significant decreases in circulating blast cells, but none had a bone marrow response (table 1).

Thrombocytopenia was observed in five of the 13 patients who received chlorozotocin weekly and in five of the seven who received the drug in the 3-day schedule, but none developed bleeding related to drug administration. Hepatic, renal, and pulmonary toxic effects were not seen. However, in this severely ill group of patients only acute rather than chronic toxic effects were evaluable.

Five of the patients with lymphoma had Hodgkin's disease and 11 had non-Hodgkin's histologies (table 2). Two patients with multiple myeloma were also included. There were two objective responses in the patients with Hodgkin's disease. One response was achieved by a patient with mixed cel-

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[3]Instituto de Investigaciones Hematologicas, Academia Nacional de Medicina, Buenos Aires, Argentina.

[4]Department of Medicine, Division of Medical Oncology, and Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC.

Reprint requests to: Paul V. Woolley, MD, Division of Medical Oncology, Georgetown University Hospital, 3800 Reservoir Rd, NW, Washington, DC 20007.

TABLE 1.—Response data for leukemia patients treated with chlorozotocin at a dose of 120 mg/m² either weekly or daily for 3 days

Diagnosis	No. of patients	No. of courses	Marrow response	Decrease in circulating blast cells
		Weekly schedule		
Acute myelocytic leukemia	5	1, 3, 3, 3, 3	0	2
Acute lymphocytic leukemia	7	1, 2, 2, 3, 3, 3, 3	0	3
Chronic myelocytic leukemia (blast cell crisis)	1	1	0	0
Total	13		0	5
		Daily × 3 schedule		
Acute myelocytic leukemia	3	1, 1, 1	0	2
Acute lymphocytic leukemia	4	1, 1, 2, 2	0	4
Total	7		0	6

TABLE 2.—Response data for lymphoma and myeloma patients treated with chlorozotocin at a dose of 120 mg/m² every 6 wks

Diagnosis	No. of patients	No. of courses	Response
Hodgkin's disease	5	1, 2, 3, 4, 5	2
Nodular lymphocytic lymphoma	2	5, 7	0
Diffuse lymphocytic lymphoma	4	1, 1, 1, 7	0
Chronic lymphocytic leukemia	3	1, 2, 3	0
Diffuse histiocytic lymphoma	2	1, 2	0
Multiple myeloma	2	3, 3	0 (slight subjective improvement and 50% decrease in serum paraprotein in 1 patient)

lularity who received three courses of drug, and the other was achieved by a patient with lymphocyte-predominant disease who received five courses of drug. The first responder had a reduction in cervical adenopathy and the second had a reduction in hilar nodes.

In the patients with non-Hodgkin's histologies (including chronic lymphocytic leukemia) there were no documented responses. Neither patient with multiple myeloma showed an objective response in terms of reduction of plasma cells in bone marrow or healing of bone lesions. One patient, however, had symptomatic improvement and a 50% decrease in serum paraprotein which lasted 6 weeks.

Drug toxicity was minimal in these patients. Most patients had no documented toxic effects, while two patients had decreases in platelet counts.

DISCUSSION

A consideration of the drug dose and schedule was important in this study of chlorozotocin in leukemias and lymphomas. In the phase I study, the dose-limiting toxicity was myelosuppression (2,3) and the recommended phase II dose was 120 mg/m² every 6 weeks, although some investigators have used doses up to 200 mg/m² every 6 weeks in phase II trials with solid tumors (4). In the present study, the dose was 120 mg/m² every 6 weeks for the lymphoma patients. For the leukemia patients, it seemed justified to use a somewhat more intensive regimen to produce marrow hypoplasia, and chlorozotocin was initially given at a dose of 120 mg/m² every week for 3 weeks. With this schedule, thrombocytopenia and some reduction in circulating blast cells were observed, but no marrow responses were noted. Subsequently, the more intensive schedule of 120 mg/m²/day for 3 days showed further evidence of antileukemic activity in terms of reductions in circulating blast cells, but produced no marrow responses. No toxic effect other than the anticipated thrombocytopenia was observed in these patients.

Two responses were observed among the lymphoma patients, both in patients with Hodgkin's disease. In the earlier phase I trial at Georgetown University (3), subjective improvement occurred in one patient with Hodgkin's disease and a partial response occurred in one patient with a nodular lymphocytic lymphoma. These data are in accord with another report of chlorozotocin activity in Hodgkin's disease (6). The data for myeloma provide a hint of activity for the drug, but more extensive evaluation of chlorozotocin in cooperative group trials will be necessary. Overall, the data indicate that while chlorozotocin is not effective in heavily pretreated leukemia and lymphoma patients, it gives evidence of activity, particularly in Hodgkin's disease, that might be exploited in a less heavily treated population.

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Chemotherapeutic Trials With Hamster Mesothelioma 10–24: Responses to Azacitidine, Aziridinylbenzoquinone, Cisplatin, and PCNU[1,2]

Brief Reports and Preliminary Communications

Stephan M. Holiat,[3] William E. Smith,[3] Doras D. Hubert,[3] and Stephen Davis[4,*]

SUMMARY

Asbestos-induced peritoneal mesothelioma 10–24 serially transplanted in Syrian golden hamsters was used to test responses to seven drugs as single agents. Compared to saline-treated controls, the average survival time increased 51%–85% with azacitidine, 42%–60% with aziridinylbenzoquinone, 43% with cisplatin, and 35%–45% with PCNU. No cures were achieved. Therapeutic responses were not observed with dactinomycin, dianhydroxyanthracenedione, or DTIC.

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In view of the poor prognosis for patients with malignant mesotheliomas, an animal model has been utilized for trials of potential chemotherapeutic agents. Responses, but no cures, were seen with cyclophosphamide, 5-FU, and doxorubicin administered as single agents (1,2). The present paper describes trials of additional drugs with this model. Responses now reported to aziridinylbenzoquinone (AZQ) and to PCNU are of interest, since those drugs are not among agents reported to have been used clinically for the treatment of mesotheliomas (3,4).

MATERIALS AND METHODS

As in previous trials with mesothelioma 10–24, animals were male Lak:LVG Syrian golden hamsters and transplants to new hosts were made by aseptic ip injection of 0.25 ml of peritoneal effusion from a tumor-bearing animal (2). Chemotherapeutic trials were carried out in groups of six hamsters each, with the following compounds supplied by the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD: dactinomycin, DTIC, PCNU, azacitidine, cisplatin, AZQ, and dianhydroxyanthracenedione.

Treatments were given by ip or im injection (table 1). Drugs were diluted for injection with 0.9% sterile saline. All treatments were started on Day 7 after transplant. All control animals received injections of sterile saline in the same schedule as the most extensively treated group. The number of days that each animal lived following transplant was recorded. In each experiment, increase in lifespan (ILS) was calculated by comparing survival time in days after transplant for treated and for control hamsters of the same transplant generation. Statistical significance was determined by Student's t test for paired comparisons. All animals were examined at necropsy.

RESULTS

Findings are detailed in table 1. In all experiments, saline control animals died within 24–38 days after transplant, with an average time of 30 days. At necropsy, these hamsters showed abundant tumor nodules on visceral and parietal surfaces, and numerous metastases in the lungs. Loss of body weight did not ensue in controls, apparently due to copious peritoneal effusions that developed.

Animals treated with azacitidine at cumulative doses of 100, 75, 60, or 40 mg/kg in schedules shown in table 1 died with extensive peritoneal mesotheliomas and pulmonary metastases, but with ILS values of 69% ($P < 0.001$), 80% ($P < 0.001$), 51% ($P < 0.2$), and 74% ($P < 0.001$), respectively. The latter response (74% ILS) was achieved when the low-

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[3]Health Research Institute, Fairleigh Dickinson University, Madison, NJ.

[4]Veterans Administration Medical Center, East Orange, NJ; and CMDNJ-New Jersey Medical School, Newark.

*Reprint requests to: Stephen Davis, MD, Oncology/Hematology, Veterans Administration Medical Center, East Orange, NJ 07019.

TABLE 1.—Chemotherapy trials with Syrian golden hamsters bearing mesothelioma 10–24 (6 hamsters/group)

Drug	Dose (mg/kg)	Route	Total dose (mg/kg)	Treatment (Days)	Survival after transplant: Average (Days)	Survival after transplant: Range (Days)	Survival after transplant: Versus control: ILS* (%)	Survival after transplant: Versus control: P value†	Body wts: At start (Day 7): Average (g)	Body wts: At start (Day 7): Range (g)	Body wts: Day	Body wts: Average (g)	Body wts: Range (g)	Wt change (%)
PCNU	15	Ip	135	7,11,14,18,21, 25,28,32,35	41	(33–49)	45	<0.002	104	(96–115)	21	102	(92–109)	−2
	30‡	Ip	60	7,14	42	(39–46)	43	<0.001	168	(146–193)	14	168	(141–184)	No change
	30‡	Ip	120	7,14,21,28	40	(35–46)	35	<0.001	153	(135–162)	28	121	(106–141)	−21
Azacitidine	50	Im	250	7,12,14,19,21	22	(20–21)	−21		91	(80–111)	19	63	(54–78)	−31
	25	Im	125	7,11,14,18,21	24	(22–26)	−16		110	(107–118)	21	85	(78–96)	−23
	25	Ip	125	7,11,14,18,21	30	(23–52)	4	NS	102	(98–105)	21	84	(78–92)	−18
	25	Ip	100	7,8,9,10	52	(46–61)	69	<0.001	103	(98–110)	22	108	(97–121)	5
	25	Im	75	7,11,18	59	(56–64)	80	<0.001	122	(122–135)	25	124	(118–134)	2
	15	Im	60	7,9,11,18	49	(22–64)	51	NS	122	(115–133)	25	96	(80–107)	−21
	10	Ip	40	7,8,9,10	54	(49–65)	74	<0.001	97	(87–105)	22	100	(89–109)	3
AZQ	4	Ip	36	7,11,14,18,21, 25,28,32,35	41	(33–51)	42	<0.005	102	(86–115)	21	108	(88–123)	6
	15‡	Ip	30	7,14	42	(41–44)	42	<0.005	149	(118–183)	14	146	(121–175)	−2
	15‡	Ip	60	7,14,21,28	47	(44–49)	60	<0.001	145	(118–181)	28	141	(120–170)	−3
Cisplatin	6	Ip	24	7,11,14,18	27	(20–41)	−5		101	(95–106)	21	60	(54–66)	−41
	1.8	Ip	9	7,8,9,10,11	45	(37–53)	43	<0.005	97	(94–102)	22	83	(60–98)	−14
DTIC	21.6	Ip	108	7,8,9,10,11	32	(30–34)	4	NS	98	(85–105)	22	99	(87–110)	1
Dianhydroxyanthracenedione	1.5	Ip	9	7,11,14,18, 21,25	27	(24–30)	−5		103	(93–110)	21	86	(75–91)	−17
Dactinomycin	0.0396	Ip	0.1980	7,8,9,10,11	25	(24–27)	−18		100	(92–106)	22	88	(71–102)	−12
Saline controls (4 groups)					30	(24–38)			99	(89–109)	21	100	(77–117)	1

*% difference in average survival time of treated group vs untreated control group of same transplant generation.
†NS = not significant.
‡3 animals/group.

est tested dose (10 mg/kg) was administered on 4 successive days. Animals treated in that manner showed stable body weights in contrast to weight losses observed for animals treated with other regimens of this drug (table 1).

A 42% ILS ($P < 0.005$) was obtained with AZQ at a dose of 4 mg/kg twice weekly, for a total of nine doses. A group of six hamsters were treated with AZQ at a dose of 15 mg/kg on Days 7 and 14. Three of these hamsters given no further treatment showed a 42% ILS ($P < 0.005$). The other three hamsters received additional doses on Days 21 and 28. They showed a 60% ILS ($P < 0.001$). The difference in ILS between these latter two groups is not significant.

Animals treated with cisplatin at a dose of 1.8 mg/kg on 5 successive days showed a 43% ILS ($P < 0.005$). Administration of this drug at a dose of 6 mg/kg on Days 7, 11, 14, and 18 was followed by deaths from extensive tumor at about the same times as controls, but drug toxicity may have been a contributing factor in these deaths since there was a 41% loss of body weight.

A group of six hamsters were given PCNU at a dose of 30 mg/kg on Days 7 and 14. Three of these hamsters received no further treatment and showed a 43% ILS ($P < 0.001$). Their body weights remained stable. The other three hamsters in this group were given additional doses on Days 21 and 28. They showed a 35% ILS ($P < 0.001$), but a 21% loss in body weights suggested cumulative drug toxicity. Treatments with PCNU at a dose of 15 mg/kg twice weekly for a total of nine doses gave 45% ILS ($P < 0.002$) with no significant change in body weights.

Treatments with DTIC, dianhydroxyanthracenedione, or dactinomycin, showed no benefit at the doses and treatment schedules listed in table 1. Animals treated with these drugs lived about as long as controls. Necropsies revealed no lesser extent of tumor in them as compared with controls.

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Reversal of Methotrexate Toxicity in Mice by a Calcium Salt of Citrovorum Factor and Related Compounds[1,2]

Brief Reports and Preliminary Communications

Carroll Temple, Jr,* Jerry D. Rose, W. Russell Laster, and John A. Montgomery[3,4]

SUMMARY

The isolation of citrovorum factor from a chemically prepared mixture of the L(±) diastereoisomers (leucovorin) is described. The natural diastereoisomer was at least twice as effective as leucovorin in reversing methotrexate toxicity in mice.

[Cancer Treat Rep 65:1117–1119, 1981]

The ability of calcium leucovorin (Lederle Laboratories) both to protect against the systemic toxicity of methotrexate (MTX) and to potentiate its antitumor activity was established by Mead et al (1), and this work led to current interest in the treatment of human cancers with high-dose MTX and citrovorum rescue (2). All of the published animal and clinical work has been done with synthetically prepared calcium leucovorin, which is a mix- of the two possible diastereoisomers resulting from chemical reduction of the pyrazine ring, both containing the L-glutamic acid residue. The natural diastereoisomer (citrovorum factor) is more effective than the mixture in promoting the growth of microorganisms (3) and in preventing the cytotoxic effects of MTX on L1210 cell growth in culture (4). These results indicate that only the natural diastereoisomer, whose absolute configuration is now known, is biologically active (5), but no in vivo studies have been reported. In this investigation, we have demonstrated that the natural diastereoisomer is more effective than the mixture in reversing MTX toxicity in mice.

MATERIALS AND METHODS

In these experiments, BDF_1 mice supplied by the Animal Genetics and Production Branch of the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, were housed in stainless steel cages (six or fewer per cage) and were given Wayne Lab Blox 6F and water ad libitum. They were given MTX ip with and without the reversal agent, which was administered sc; the doses were based on the exact body weight of each individual animal. All compounds were given as a single dose, and the mice were observed for 30 days after treatment.

To produce Ca L(–)-5-formyl-5,6,7,8-tetrahydrofolate (calcium salt of citrovorum factor, CaCF), crude Ca 5-CHO-THF (120 g), was purified by Florisil column chromatography: yield, 70.4 g (6). This sample was extracted at room temperature with oxygen-free H_2O (2 × 1100 ml), and the combined extracts were lyophilized to give Ca 5-CHO-THF: yield, 58.9 g; $[\alpha]_D^{25} = +16.0 \pm 0.4°$ (c = 1% wt/vol, H_2O). In reference 3, $[\alpha]_D = +14.26°$ (3.42%, H_2O).

The insoluble residue was dried in vacuo over P_2O_5: yield, 9.50 g. $[\alpha]_D^{25} = -13.0 \pm 0.4°$ (c = 1% wt/vol, H_2O).[5]

The analysis calculated for $C_{20}H_{21}N_7O_7 \cdot Ca \cdot 1.5H_2O$ (molecular weight, 538.5): C, 44.61; H, 4.49; N, 18.21; Ca, 7.44; and ash (CaO), 10.58. The analysis found: C, 44.50; H, 4.38; N, 18.22; Ca, 7.56; and ash (CaO), 10.80.

A portion (6.3 g) of the residue was heated at 50°C in deaerated H_2O (425 ml), and some insoluble material (91 mg) was removed by filtration under

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[3]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, AL.

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*Reprint requests to: Dr. Carroll Temple, Jr, Organic Chemistry Research Department, Southern Research Institute, 2000 Ninth Ave S, Birmingham, AL 35255.

[5]Concentration based on anhydrous CaCF.

N_2. The clear yellow filtrate was cooled to room temperature and chilled in an ice bath for 1 hour; the solid that deposited was collected by filtration and dried in vacuo over P_2O_5: yield, 2.1 g (sample A). $[\alpha]_D^{25} = -25.2 \pm 0.4°$ (c = 0.91% wt/vol, H_2O). In reference 3, $[\alpha]_D = -15.1°$ (c = 1.82%, H_2O).

The analysis calculated for $C_{20}H_{21}N_7O_7 \cdot 1.25Ca \cdot 4H_2O$ (molecular weight, 593.6): C, 40.47; H, 4.92; N, 16.52; Ca, 8.44; and ash (CaO), 11.81. The analysis found: C, 40.85; H, 4.78; N, 16.63; Ca, 8.51; and ash (CaO), 12.44.

After storage at –5°C for 1 year, a fresh solution of sample A in H_2O was opaque in a polarimeter (254 nm). After standing for 20 hours, however, this solution gave $[\alpha]_D^{25} = -20.5 \pm 1.1°$ (c = 1.0% wt/vol, H_2O). Apparently, the initial solution contained dispersed but undissolved material, which settled; and the upper, clear portion of this solution gave a rotation. These results suggested that only slight decomposition of sample A had occurred during storage, which was confirmed by the high-pressure liquid chromatogram of the solution. This sample A and a commercial sample of Ca L(±)-CF (Lederle Laboratories) were analyzed for relative potency, using *Pediococcus cerevisiae* and a broth dilution assay (6). Three separate analyses carried out on each sample, using separate weighings, showed that sample A had a potency relative to Ca L(±)-CF of 1.96 ± 0.16 (SD), ±0.090 (SE). These results suggest that sample A is the practically pure, natural diastereoisomer (citrovorum factor) of Ca 5-CHO-THF.

RESULTS AND DISCUSSION

In the preparation of a large amount of calcium leucovorin by an improved procedure (7), we isolated a sample of calcium salt having an optical rotation similar to that reported for citrovorum factor (3). Recrystallization of this material gave a sample with an optical rotation, $[\alpha]_D^{25}$, of $-25.2 \pm 0.4°$, a negative value larger than that previously reported for chemically prepared citrovorum factor (3) and similar to that recently reported for enzymatically prepared citrovorum factor (8). This material was shown to be twice as potent in supporting the growth of *Pediococcus cerevisiae* as the racemic mixture, indicating that it was the biologically active diastereoisomer; recrystallization did not increase its negative rotation.

The results of two separate experiments on the reversal of MTX toxicity are given in table 1. The doses of calcium leucovorin and citrovorum factor were given 6 hours after the supralethal dose of MTX. Semilog plots of dose versus percent survivors, using a least-squares fit of the data points, gave essentially parallel lines and an ED50 value of 100 mg/kg for leucovorin and 30 mg/kg, or about one third of the value for leucovorin, for the synthetic citrovorum factor. These results agree well with recently reported in vitro reversal studies comparing citrovorum factor and leucovorin (4).

L(±)-5,10-methenyl-5,6,7,8-tetrahydrofolic acid chloride, an intermediate in the preparation of the 5-formyl compound (6), and Ca L(±)-5-methyl-5,6,7,8-tetrahydrofolate were also effective in reversing the toxicity of MTX, whereas Ca 10-formyl-7,8-dihydrofolate, containing Ca 10-formylfolate (2:1) (9) and an analog, Ca N^5,N^{10}-imidocarbonyl-5,6,7,8-tetrahydrofolate (10), were almost ineffective (table 2).

These results indicate not only that a tetrahydrofolate is necessary for reversal but also that the tetrahydrofolate must be capable of transformation by the folate enzymes. In addition, the efficacy of the natural diastereoisomer was more than twofold

TABLE 1.—Reversal of MTX toxicity by leucovorin and citrovorum factor*

Compound	Dose (mg/kg)	% survivors	
		Experiment 1	Experiment 2
None	—	0	0
Calcium leucovorin	200	50	83
	100	0	67
	50	33	17
	25	17	0
	12.5	0	0
Citrovorum factor	200	100	100
	100	100	83
	50	50	33
	25	17	67
	12.5	67	17

*6 animals were given a single dose of 1000 mg/kg of MTX: males were used in experiment 1, and females were used in experiment 2.

TABLE 2.—Reversal of MTX toxicity by leucovorin analogs

Compound*	Dose (mg/kg)	% survivors		
		500 mg of MTX	1000 mg of MTX	1500 mg of MTX
None	—	0	0	0
Leucovorin	500	100	80	40
	337	100	80	10
	225†	100	70	0
	150	80	70	10
5,10-Methenyl	500	30	20	0
	337	70	20	0
	225	40	10	0
	150	50	0	10
5,10=C=NH	500	10	0	0
	337	0	0	0
	225	20	0	0
	150	0	0	0
10-CHO-7,8-diH	500	0	0	0
	337	30	0	0
	225	10	0	0
	150	0	0	0
5-Me‡	300	100		
	200	50		
	100	83		
	50	10		

*Unless otherwise indicated, 10 female mice were given each of these compounds.

†9 mice were given this dose of leucovorin.

‡6 mice were given this compound.

greater than that of the mixture of diastereoisomers in the reversal of MTX toxicity in mice. This result might be attributed either to a detrimental effect of the unnatural diastereoisomer on reversal or to a sample of calcium leucovorin containing < 50% of the natural diastereoisomer. Studies involving purified material will be necessary to determine the biologic effect, if any, of the unnatural diastereoisomer.

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Cisplatin-Induced Anemia

Recent reports (1–3) concerning cisplatin-induced anemia have prompted us to report the following.

A 14-year-old Latin American male with osteosarcoma started treatment with cisplatin at a dose of 150 mg/m² (actual dose, 200 mg) and mannitol diuresis, alternating with doxorubicin (75–90 mg/m²) at 3-week intervals. The first course of cisplatin was administered in February without incident. The hemoglobin, bilirubin, and platelet counts at that stage were 10.7 g/100 ml, 0.3 mg/100 ml, and 418,000/mm³, respectively. Before the second course of cisplatin was administered in March, the direct Coombs' test was reported as negative, and the hemoglobin and serum bilirubin were 9.3 g/100 ml and 0.2 mg/100 ml, respectively. Soon after the infusion, the patient developed moderate scleral icterus. Laboratory studies revealed hyperbilirubinemia (total, 5.7 mg/100 ml; indirect, 3.1 mg/100 ml) and a hemoglobin of 8.9 g/100 ml. There was a progressive decrease in the platelet count from 588,000 to 180,000/mm³ over the ensuing 2 weeks. Of major interest, however, was the detection of a positive Coombs' test 12 hours after cisplatin infusion, with negative anti-IgG and anti-C3.

At 48 hours the direct Coombs' test was again positive. The anti-IgG was negative and the anti-C3 was positive. Hepatitis-associated antigen was negative. Fourteen days after cisplatin infusion, the direct Coombs' test and the anti-C3 were still micropositive and the anti-IgG was negative. The patient was retested 6 weeks later at which stage the direct Coombs' test was negative. Because he was considered to be at high risk for the development of pulmonary metastases, cisplatin was again administered with informed consent. Twelve hours later, the direct Coombs' test was again positive, the anti-IgG was micropositive, and the anti-C3 was negative. Three days later, the direct Coombs' test, anti-IgG, and anti-C3 were micropositive. The hemoglobin, serum bilirubin, and platelet counts were similar to pretreatment values.

The patient has subsequently tolerated two additional courses of cisplatin without overt evidence of hemolysis, although the direct Coombs' test remains weakly positive. Review of the effects of > 50 cisplatin infusions in other patients has failed to demonstrate similar episodes of hyperbilirubinemia and anemia. In several patients, the direct Coombs' tests were performed and the results were negative.

Our case report supports the observations recorded by Getaz et al (1,3). It also indicates that rbc sensitization induced by cisplatin may be asymptomatic and mild and its presence may only be suspected by means of a positive Coombs' test. In addition, hemolysis does not necessarily preclude additional cisplatin treatment, although further episodes of a more severe nature must be considered a potential threat. Additional study is required to determine the incidence and severity of cisplatin-induced hemolysis.

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Bay Van Nguyen
Norman Jaffe

Department of Pediatrics

Benjamin Lichtiger

Department of Laboratory Medicine

The University of Texas System
Cancer Center
M. D. Anderson Hospital and
Tumor Institute
6723 Bertner Ave
Houston, TX 77030

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Cardiac Arrhythmia Associated With AMSA in a Child: A Southwest Oncology Group Study [1]

AMSA is a new agent being used in the treatment of selected solid tumors and acute leukemias. There has been a report of both neurologic and cardiac toxic effects in adults (1). An additional case of fatal ventricular fibrillation during AMSA infusion has been reported (2). To date, eight documented cases of cardiotoxicity associated with the use of AMSA have been reported to the National Cancer Institute, Bethesda, MD (S. Marsoni, personal communication, 1980). This case report describes a child who developed cardiac arrest followed by ventric-

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ular fibrillation, temporarily associated with the administration of AMSA.

CASE REPORT

The patient, a white female child aged 9 years and 4 months, had been diagnosed as having acute lymphocytic leukemia at the age of 1 year and 11 months. She had experienced five CNS and nine bone marrow relapses, including the marrow relapse for which she was to receive AMSA. Her prior treatment included multiple combinations of chemotherapy including doxorubicin (cumulative dose, 435 mg/m²). She was admitted to start AMSA using the Southwest Oncology Group pediatric protocol (SWOG-7919) for this investigational drug. AMSA was to be given at tae dose of 60 mg/m²/day iv for 10 days.

The child was emaciated and had alopecia. Heart examination showed a soft grade I/VI systolic ejection murmur along the left sternal border; the murmur did not radiate and had no associated thrill. Pulses were normal. CNS examination demonstrated bilaterally depressed reflexes. Examination of her cerebrospinal fluid revealed no evidence of leukemia. Chest roentgenogram and echocardiogram were normal. Biochemical profile revealed Na = 140 mEq/liter, K = 2.6 mEq/liter (normal range, 3.5–5 mEq/liter), Cl = 102 mEq/liter, CO_2 = 26 mEq/liter, BUN = 3 mg/100 ml, Ca = 8.7 mg/100 ml, and creatinine = 0.6 mg/100 ml.

The patient was given 60 mg of AMSA diluted in 100 ml of 5% dextrose in water over 1 hour without difficulty. Twenty minutes after the infusion, she was found to be unresponsive, with no audible heartbeat or palpable pulses. Within 2 minutes, cardiopulmonary resuscitation was started. The patient was intubated and received chest compressions. A cardiac monitor revealed coarse ventricular fibrillation. The patient was cardioconverted at 200 W/second to a normal sinus rhythm on the fourth attempt. Her pupils remained reactive during the 40 minutes of cardiopulmonary resuscitation. She was transported to the intensive care unit in an awake and responsive state. ECG after the arrest showed signs of electrolyte disturbance, although several determinations of serum potassium level were > 2.6 mEq/liter.

The patient's remaining hospital course was unremarkable. There were no signs of any neurologic or ischemic myocardial damage secondary to her cardiopulmonary arrest. A followup echocardiogram showed evidence of increased left ventricular size (increased end-diastolic and end-systolic dimensions) with decreased contractility. She also had radiographic evidence of aspiration pneumonia. She was discharged 10 days after the episode; her cardiac examination was normal when she was discharged. She subsequently died 29 days following AMSA infusion; autopsy was denied.

DISCUSSION

Although cardiac toxic effects have not been found to be a complication in experimental animals (3), they have been noted in man (1,2). In three adult patients, hypokalemia (K^+ = 1.9, 2.3, and 2.7 mEq/liter) appeared to be associated with cardiac arrhythmia. Our patient's potassium was 2.6 mEq/liter at the time of her ventricular fibrillation. Although a causal role cannot be directly attributed to the AMSA because of electrolyte disturbance, AMSA may stimulate arrhythmias in a sensitized myocardium. A recent report documented ECG and echocardiographic abnormalities in children receiving AMSA after previous exposure to anthracyclines (4). A post-AMSA echocardiogram revealed decreased contractility compared to the pre-AMSA studies. There may be an association of subclinical cardiotoxicity with the previous administration of anthracyclines, which may predispose the myocardium to develop arrhythmias with AMSA infusions, especially in the presence of hypokalemia. We recommend that electrolytes be carefully monitored and correction of any abnormalities be made before administering AMSA. Also, cardiac monitoring for any arrhythmias during and immediately following AMSA administration is advised.

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Anthony R. Riela*
Jim C. Kimball
Richard B. Patterson

Bowman Gray School of Medicine
Winston-Salem, NC

Vita J. Land

Washington University
St. Louis, MO

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*Reprint requests to: Dr. Anthony R. Riela, Pediatric Oncology Group Operations Office, 2nd Floor, 4386 Lindell Blvd, St. Louis, MO 63108.

5-FU Cardiotoxicity

Cardiotoxicity is a well-known side effect of the cytotoxic anthracyclines doxorubicin and daunorubicin (1), but it is relatively unknown as a side effect of 5-FU. We are reporting two cases of such toxicity with ECG or cardiac enzyme changes that were noted while 5-FU was being administered. Patient 1 received 5-FU infusion with concurrent radiation therapy to the thorax for a squamous cell carcinoma of the left upper lobe (LUL). On Day 4 of a 5-day infusion course, he developed chest pain with ECG changes. Patient 2 developed chest pain and ECG changes in his second course of concurrent 5-FU infusion and radiation therapy to the rectum for recurrent carcinoma. In his third course of treatment, he again developed chest pain, followed by a cardiac arrest.

CASE REPORT

Patient 1

Patient 1, a 64-year-old white male with inoperable squamous cell carcinoma of the LUL, had no past history of diabetes mellitus, hypertension, angina pectoris, or myocardial infarction. He was admitted on March 24, 1980, for treatment with 5-FU given by continuous infusion (20 mg/kg/day) on Days 1–5 and radiation therapy (250 rads) to the LUL, mediastinum, and supraclavicular area on Days 2–5. On Day 4 of his first course, after receiving a total dose of 7200 mg of 5-FU and 750 rads of radiation he developed dull chest pain, radiating to his arms and left shoulder. The ECG showed ST elevations in leads II, III, and AVF, with ST depression in AVR.

The patient was transferred to the coronary care unit for further observation. No enzyme changes were detected and the ECG returned to normal. The subsequent 5-FU infusion and irradiation were carried out with a vasodilator (Nitropaste, 1 inch every 6 hours) as a preventive maneuver. There were no further episodes of chest pain with further 5-FU infusion and radiation therapy. This patient subsequently had a normal stress test and normal coronary angiography.

Patient 2

Patient 2, a 59-year-old black male, was admitted with rectal pain secondary to biopsy-proven recurrence of adenocarcinoma of the rectum (stage 1 bronchoalveolar carcinoma of the right upper lobe previously resected). His past history was negative for diabetes mellitus, hypertension, angina, and myocardial infarction. 5-FU infusion (20 mg/kg/day) and radiation therapy to the rectum was started; the combination was to be given every other week to a total radiation dose of 5000 rads.

The first week of therapy was uneventful. Following a 1-week rest period, he returned for his second course of 5-FU and radiation therapy. On Day 4, he developed precordial chest pain. His ECG showed inverted T waves in V_4–V_6 and lead I. No cardiac enzyme changes were detected, and his ECG returned to normal when the 5-FU infusion was stopped (figs 1A and B). Nevertheless, Nitropaste (1 inch every 6 hours) was started as a preventive maneuver. He had no chest pain during the next rest period.

He was readmitted on April 28, 1980, for the third course of treatment. On Day 4, he developed severe retrosternal chest pain, and the ECG showed ST elevation in leads II, III, and AVF; ST depression in V_1–V_2 (fig 1C); and inverted T wave in AVL. The patient had ventricular tachycardia and cardiac arrest and was successfully resuscitated by cardioversion. Cardiac enzyme determinations indicated myocardial injury (previously normal LDH, SGOT, and CPK increased to 660, 300, and 1111 IU/liter, respectively). The ECG returned to normal and he recovered without further complications.

DISCUSSION

5-FU has been available as a cytotoxic agent since 1957 (2). Today, it is predominantly used in the treatment of patients with adenocarcinoma of the digestive tract (stomach, pancreas, liver, and colon) and carcinoma of the bladder, ovary, and breast. The toxic effects of 5-FU are well-described and include myelosuppression, nausea, vomiting, stomatitis, alopecia, and cerebellar ataxia (3). Al-

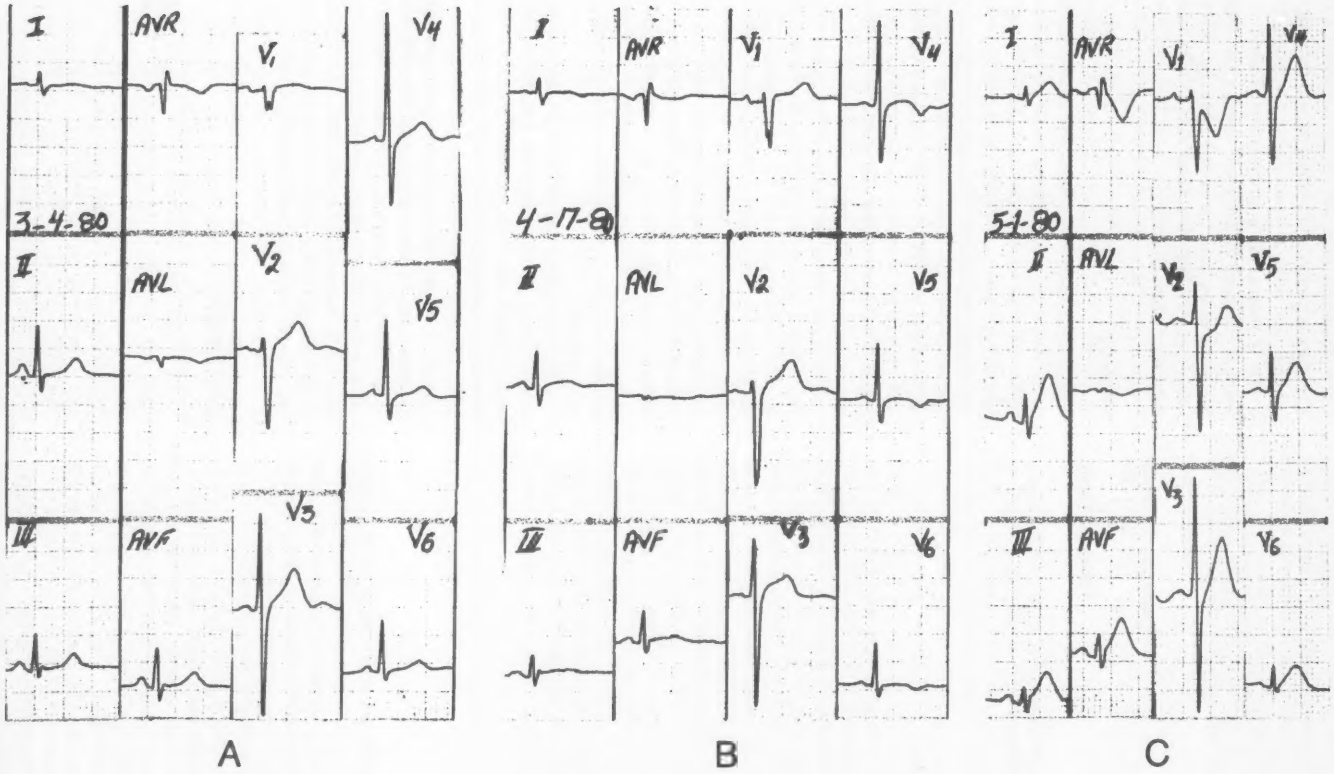

FIGURE 1.—A: normal ECG; B: ECG changes (V_4–V_6) during 5-FU infusion; and C: ECG changes in second challenge.

though there have been a few scattered reports of chest pain associated with the administration of 5-FU, these are very rare in comparison with the frequency with which the drug is administered.

The 12 previously reported cases (4–6) have certain features in common. All of the patients developed chest pain after prolonged infusion of 5-FU or repeated bolus courses; all had ECG changes which reversed to normal when the 5-FU was stopped; only one had cardiac enzyme changes (6) (subendocardial infarct); and only one had had a previous infarct, 2 years prior to treatment (case 1, ref 4). There was no other history of hypertension, angina, or myocardial infarct in this group.

We would like to add two more cases to the series. Both of our patients developed chest pain and ECG changes while they were receiving 5-FU by prolonged infusion. Neither had a previous history suggestive of cardiac disease.

The cause of 5-FU cardiotoxicity is unknown, but numerous etiologies have been suggested, including coronary artery spasm due to 5-FU, pulmonary hypertension caused by spasm in the pulmonary arteries, changes in platelets resulting in increased aggregability, or direct toxic effect of 5-FU on myocardial cells, similar to that seen with doxorubicin. We believe that coronary artery spasm is the most likely cause since the majority of episodes were transient and occurred in patients without previous cardiac problems. Moreover, our first patient had normal coronary angiography following his chest pain. Further studies are indicated, however, to confirm and to investigate the apparent cardiotoxicity of 5-FU since it is being used more and more in combination with other cytotoxic agents and with irradiation.

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Shamel Sanani*
Monica B. Spaulding
A. R. Zaki Masud
Ruth Canty[1]

February 23, 1981

[1]Oncology Section, Veterans Administration Medical Center, (*S. Sanani, M. B. Spaulding*, and *R. Canty*) and Department of Medicine, State University of New York at Buffalo, (*S. Sanani, M. B. Spaulding*, and *A. R. Z. Masud*), Buffalo, NY.

**Reprint requests to:* Shamel Sanani, MD, Veterans Administration Medical Center, Oncology Section, 3495 Bailey Ave, Buffalo, NY 14215.

Concomitant Phase II Studies of Pyrazofurin and Razoxane in Alkylating Agent-Resistant Cases of Epithelial Ovarian Carcinoma[1,2]

Pyrazofurin inhibits DNA replication by interfering with orotodylic acid decarboxylase (1). This agent has been found to be tolerable in iv doses of 240 mg/m^2 given every 4 weeks (2). Razoxane inhibits mitosis during the G_2-M phase of the cell cycle (3,4). This agent has been found to be tolerable in divided oral doses of 750 mg/m^2 daily for 3 consecutive days every 4 weeks (5).

All of the 27 patients involved in these studies had experienced objective progression of histologically proved stage III or IV epithelial ovarian carcinoma while receiving at least one alkylating agent, and all except four had received doxorubicin before entering these phase II trials. Before beginning a

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[2]Pyrazofurin and razoxane were supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

TABLE 1.—Phase II study of pyrazofurin in advanced ovarian carcinoma

Age (yrs)	ECOG performance status	Previous treatments*	Best pyrazofurin response	No. of courses of pyrazofurin	Toxic effects
57	3	Radiation to abdomen, thioTEPA, CTX-ADR	Disease progression	1	Data insufficient
58	1	CTX, CTX-ADR, razoxane	Not evaluable (patient refused to return)	1	Data insufficient
39	0	Radiation to abdomen and pelvis, CTX, melphalan, ADR	Disease progression	1	None recognized
51	1	CTX	Stable	2	Mucositis, dermatitis
56	0	Radiation to pelvis, CTX-ADR	Stable	10	Mucositis, nausea and vomiting, leukopenia (3900 cells/μl)
42	0	Radiation to pelvis, CTX, CTX-ADR, razoxane	Disease progression	1	Mucositis, diarrhea, nausea and vomiting, leukopenia (3800 cells/μl)
64	2	CTX-ADR, CTX	Stable	3	Mucositis, dermatitis, leukopenia (3800 cells/μl), thrombocytopenia (85,000 cells/μl)
43	2	CTX, CTX-ADR	Disease progression	1	Nausea and vomiting, leukopenia (3400 cells/μl)
70	1	Radiation to abdomen and pelvis, chlorambucil, CTX-ADR, CTX	Disease progression	3	Nausea and vomiting, dermatitis
69	1	CTX, CTX-ADR	Stable	3	Mucositis, nausea and vomiting, diarrhea
67	3	CTX-ADR, CTX	Stable	6	Nausea and vomiting, diarrhea, mucositis, leukopenia (2000 cells/μl), thrombocytopenia (51,000 cells/μl)
64	1	CTX-ADR, CTX, rozoxane	Disease progression	2	Nausea and vomiting
51	2	CTX, CTX-ADR	Disease progression	2	Mucositis
49	2	CTX-ADR, CTX	Stable	2†	Mucositis, dermatitis
50	2	CTX-ADR, CTX, razoxane	Disease progression	1	Data insufficient
57	1	CTX, CTX-ADR	Disease progression	1	Data insufficient
68	1	CTX-ADR-5FU, CTX	Disease progression	2	Nausea and vomiting, stomatitis
68	1	CTX-ADR, CTX	Stable	4	Nausea and vomiting, alopecia, leukopenia (3500 cells/μl)
67	2	Radiation to pelvis, CTX-ADR, CTX	Stable	3	Mucositis, nausea and vomiting

*CTX = cyclophosphamide, ADR = doxorubicin.

†This patient received 95 mg/m^2 daily for 3 consecutive doses early in our study.

TABLE 2.—Phase II study of razoxane in advanced ovarian carcinoma

Age (yrs)	ECOG performance status	Previous treatments*	Best razoxane response	No. of courses of razoxane	Toxic effects
58	1	CTX, CTX-ADR	Stable	7	Leukopenia (1600 cells/μl), alopecia, diarrhea, stomatitis
55	3	Pelvic radiation, CTX, Depo Provera, ADR, chlorambucil	Treatment failure due to toxicity	1	Reactivation of hemorrhagic cystitis
51	3	CTX, PRZF	Disease progression	1	Data insufficient
58	0	Pelvic radiation, CTX-ADR, PRZF	Stable	3	Leukopenia (2200 cells/μl), nausea and vomiting, diarrhea
42	0	Pelvic radiation, CTX, CTX-ADR	Stable	4	Leukopenia (2100 cells/μl), thrombocytopenia (60,000 cells/μl)
53	2	CTX-ADR	Disease progression	1	Leukopenia (2100 cells/μl)
70	2	Radiation to abdomen and pelvis, chlorambucil, CTX-ADR, CTX, PRZF	Stable	2	Leukopenia (1100 cells/μl)
58	3	CTX, ADR, melphalan	Stable	2	Leukopenia (1300 cells/μl), nausea and vomiting
72	1	CTX	Not evaluable	1 (incomplete)	Nausea and vomiting, early unexpected death (only 1/3 of dose given)
56	1	CTX, megestrol acetate	Disease progression	2	Leukopenia (800 cells/μl), nausea and vomiting, thrombocytopenia (19,000 cells/μl)
58	2	CTX, ADR	Stable	4	Leukopenia (1200 cells/μl), nausea and vomiting, diarrhea
64	1	CTX-ADR, CTX	Stable	4	Leukopenia (2700 cells/μl), nausea and vomiting
70	2	CTX-ADR	Treatment failure due to toxicity	1	Mucositis, alopecia, dermatitis
50	0	CTX-ADR, CTX	Stable	2	Nausea and vomiting
36	1	CTX	Disease progression	2	Nausea and vomiting, leukopenia (1100 cells/μl)
67	3	CTX-ADR, CTX, PRZF	Disease progression	1	Data insufficient
67	2	Radiation to pelvis, CTX-ADR, CTX, PRZF	Disease progression	2	Leukopenia (1100 cells/μl), thrombocytopenia (2500 cells/μl)

*CTX = cyclophosphamide, ADR = doxorubicin, PRZF = pyrazofurin.

treatment trial, each patient was required to have evaluable disease, estimated survival of at least 2 months, and adequate renal, hepatic, and bone marrow function.

Fifteen patients were initially assigned at random to receive pyrazofurin and 12 to receive razoxane. After failure of pyrazofurin, five patients qualified for and received razoxane trials, and following initial failure of razoxane four patients qualified for and received pyrazofurin trials for a total phase II experience of 19 pyrazofurin patient trials and 17 razoxane patient trials. An attempt was made to give at least two consecutive monthly courses of pyrazofurin or razoxane in each individual trial using the regimens described above with appropriate dose modifications for severe myelosuppression, mucositis, or dermatitis.

No objective tumor regressions occurred. Of the 19 patient trials of pyrazofurin, disease progression occurred before the second monthly return visit in ten cases and disease stability was achieved for periods of from 2 to 9 months in eight others (table 1). Of the 17 patient trials of razoxane, disease progression occurred in six and treatment failure due to toxicity occurred in two others before the second monthly return visit. Disease stability was achieved for periods of from 2 to 6 months in eight patients (table 2).

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J. H. Edmonson

Division of Medical Oncology

D. G. Decker
G. D. Malkasian
M. J. Webb

Department of Obstetrics and Gynecology

Mayo Clinic
Rochester, MN 55901

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Phase II Study of PCNU in Colorectal Carcinoma[1]

PCNU is a new chloroethylnitrosourea in clinical trial. Its lipid solubility characteristics and high alkylating activity are regarded as features that could favor clinical activity (1,2). This report describes a phase II study of PCNU in advanced colorectal carcinoma.

Between January 1980 and February 1981 34 patients with advanced, histologically proven adenocarcinoma of the colon or rectum were treated with PCNU. Characteristics of the patient population are shown in table 1. A total of 14 patients had received no prior therapy, and 18 had received prior chemotherapy, including 11 who had received nitrosoureas. All patients had an Eastern Cooperative Oncology Group performance status ≤ 3 (median, 1) and all had at least one site of disease that could be used as a measure of objective response. These sites were abdominal mass (five patients), liver (20), lung (four), and liver plus lung (five). The PCNU dose was 100 mg/m² in previously untreated and 75 mg/m² in previously treated patients. It was administered as a 30-minute iv infusion every 6 weeks.

TABLE 1.—Patient characteristics

	No. of patients
Patients in study	34
Male	22
Female	12
Age (yrs)	
Median: 64	
Range: 37–80	
Prior therapy	
None	14
Chemotherapy + radiotherapy	4
Radiotherapy alone	2
Chemotherapy alone	14
Nitrosourea	11

RESULTS AND DISCUSSION

No clinical responses were observed in the 34 patients treated in this study. Progressive disease was usually apparent after the first course, and only 12 patients received more than one dose. The principal toxic reaction was thrombocytopenia. Twelve patients developed platelet counts < 100,000/mm³, with five < 60,000 and two < 25,000/mm³. Two patients, one with no prior chemotherapy, had sustained thrombocytopenia lasting > 10 weeks following therapy. There were no bleeding complications. Leukopenia was less severe, with only six patients developing wbc counts ≤ 3000/mm³; none were sustained and no infectious complications occurred. Gastrointestinal side effects were minimal, with nausea reported in ten patients and vomiting in five.

In general, nitrosoureas such as BCNU and methyl-CCNU have marginal (10%–20%) single-agent activity in colon carcinoma (3). We conclude from this study that PCNU lacks meaningful activity in this disease. The patient study group is one with favorable clinical features, namely good performance status and, in 14 cases, no prior chemotherapy. The drug has been used at a level close to the maximal tolerated dose of 105 mg/m² (4,5; PV Woolley, PVT Luc, A Rahman, et al, manuscript submitted) and significant bone marrow toxicity has been observed in this group. Hence, the failure to observe responses presumably reflects absence of drug activity rather than the presence of significant unfavorable clinical patient characteristics. Since we have worked at a dose close to the maximum tolerated, it is unlikely that further dose escalation would lead to greater therapeutic benefit.

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Edith P. Mitchell
John Y. Killen
Frederick P. Smith

Linda L. Willis
Philip S. Schein
Paul V. Woolley[2,*]

Department of Medicine
Division of Medical Oncology
Vincent T. Lombardi Cancer Research Center
Georgetown University Medical Center
3800 Reservoir Rd, NW
Rm 2100 W
Washington, DC 20007

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*To whom reprint requests should be sent.

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Fourth Annual Bristol-Myers Symposium on Cancer Research: Tumor Cell Heterogeneity—Biologic and Clinical Implications

Baltimore, Maryland, December 3–4, 1981

This symposium will be held in the Turner Auditorium at the Johns Hopkins Medical Institution in Baltimore, MD. Noted investigators from North America and abroad will review the observations that led to the conclusion that cellular heterogeneity is a common characteristic of many cancers. They will consider the genetic and epigenetic mechanisms probably responsible for the changing cellular composition of tumors. The symposium will focus on the broader biologic implications of the work under discussion and the potential relevance of the research findings to the improved management of neoplastic diseases.

No registration fee is required for attending the symposium. Attendance will be limited and application for participation will be answered in order of receipt. CME credits will be awarded.

For further information contact: Mrs. Ellie Trowbridge, Symposium Coordinator, Rm 169, Johns Hopkins Oncology Center, 600 N Wolfe St, Baltimore, MD 21205 (301–955-2583).

Sixty-fourth Annual Meeting of the American Radium Society

San Antonio, Texas, March 14–18, 1982

The American Radium Society will hold its annual meeting at the Hilton Palacio Del Rio in San Antonio, Texas, on March 14–18, 1982. *For information contact:* Mrs. Sally Polek, Office of the Secretariat, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (215-574-3181).

Sixth Annual Meeting of the Cell Kinetics Society

Houston, Texas, March 18–21, 1981

The sixth annual meeting of the Cell Kinetics Society will be held at the Dunfey Houston Hotel, Houston, Texas, on March 18–21, 1982. The Cell Kinetics Society has an international membership of approximately 300 experimentalists, clinicians, and mathematical modelers who share an interest in the cell proliferation patterns of normal and abnormal tissues.

Inquiries concerning this meeting or membership in the Society should be directed to: Dr. Bruce F. Kimler, Secretary, Cell Kinetics Society, Department of Radiation Therapy, University of Kansas Medical Center, Rainbow Blvd at 39th St, Kansas City, KS 66103.

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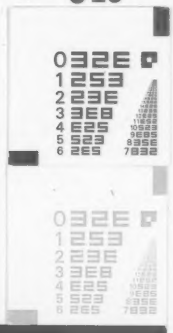
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